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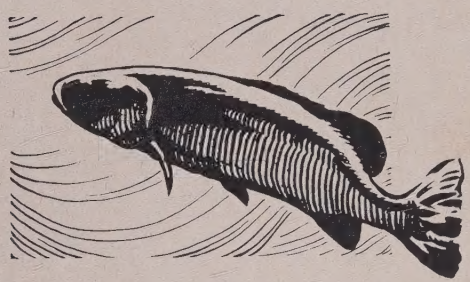

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LAND RECLAMATION AND FISH CULTURE IN THE DELTAIC AREAS OF WEST BENGAL, INDIA

T. V. R. Pillay
Central Inland Fisheries Research Station
Calcutta, India

THE DELTAIC AREA OF THE RIVER GANGES IN WEST BENGAL consists of numerous islands interspersed with a network of tidal streams of varying sizes, overgrown in many instances with dense jungles and forests. This region, generally known as Sunderbans, abounds in low, swampy lands which are completely inundated during high tides and partially or fully exposed during low tides. By the rapid silting of creeks, tidal streams, and tributaries of rivers, or by the changing of their courses, additional low, marshy lands are constantly being formed in the Sunderbans.

Because of the increasing pressure on cultivated land in West Bengal and the need to increase food production in the State, the reclamation of the Sunderbans has received attention since very early times. The reclamation of these areas involves the raising of the level of the land and the removal of the salt content of the soil to make it suitable for the cultivation of rice. Because of the large outlay required for the quick conversion of these lands into ricefields, a slow process of reclamation--which involves less expenditure and enables the production of appreciable quantities of fish during the reclamation period--has been evolved. Many fields, after being reclaimed, are utilized for the culture of fish along with rice. Hora and Nair (1944), Pillay (1954), and Pillay and Bose (in press) studied certain aspects of the fish-cultural practices; and further detailed investigations are now in progress in the Central Inland Fisheries Research Station, with a view to evolving suitable methods for increasing fish production from these waters. As the existing practices--even though capable of great improvement--are likely to be of interest to countries where such large-scale reclamation of swampy areas is contemplated, their salient features are briefly described in this article.

Reclamation of Saline Swamps

The first step in the reclamation of the saline swamps in Sunderbans consists of the construction of a strong, high embankment all round the land to prevent the uncontrolled flooding of the area with tidal water. This embankment is made with earth and mud obtained by digging a canal around the site. Generally the embankment measures 3.0 to 3.7 meters in width at the base, 1.8 to 2.1 meters in width at the crown, and 1.8 to 2.4 meters in height. On the side facing the estuary or other water source, a sluice gate is installed in the embankment to regulate the ingress and egress of water. Every day during high tides the sluice gate is opened and tidal water is taken in. When the tide turns, the sluice gate is closed to prevent the water from draining.

Throughout the year the tidal water contains very appreciable quantities of suspended silt and a good part of the silt settles in the embanked swamp (locally known as bheri). In the course of a few

A typical saline swamp suitable for reclamation.

years, the level of the bheri gets raised by the deposition of silt to the extent required for rice cultivation. But, to render the land suitable for cultivation, the salt content of the soil has to be removed. For this purpose, taking in of tidal water is stopped and the brackish water in the bheri is drained at low tides. Then, during the monsoons, rainwater is accumulated in the bheri and allowed to stand for some time, after which it is drained through the sluice gate at regular intervals. When the land is thus repeatedly flushed with rainwater, the major portion of the salt content in the soil is washed out and the soil becomes suitable for cultivation of rice, especially varieties that can withstand low salt content of soil.

Culture of Fish During Reclamation

The reclamation of saline swamps in the manner described here would take 15 to 20 years to complete, and during this period the farmer ordinarily would not get any return for his investment -- except, perhaps, some little money that he might make by selling the firewood obtained during the clearance of jungles on the lands. Consequently, there has been developed in the bheris a system of fish culture which has proved very profitable to the farmer--so much so that many farmers often prefer to maintain the areas as fish farms instead of converting them into ricefields. Only slight modifications in the reclamation processes and the installation of a few inexpensive protective devices are required to utilize the bheris for fish culture. Besides the main canal all round the embanked area, a network of smaller canals generally is dug across the land under reclamation to enable easy drainage of water. These canals form very suitable refuges for the cultivated fish. To prevent the escape of fish from the bheri, V- or W-shaped walls of bamboo grating are erected in front of the sluice gate. At the apex of the V or apices of the W, traps are set to capture fish that swim against the current when tidal water is taken.

When the embanked area is to be utilized for fish culture, the taking of tidal water is stopped by September or October and as much water as possible is drained from the area. The land is then allowed to dry. It is believed that the fertility of the soil and thereby the production of benthic food organisms for fish will be increased appreciably by the exposure of the soil. From January onward, when the tidal water in the estuary contains juveniles of certain species of grey mullet (Mugil), tidal water is taken regularly into the bheri and the screen of bamboo grating at the sluice prevents the escape of young fish that enter. Tidal water is regularly taken in every day at high tide, if possible, or only during the spring tides in areas where the tidal height is low. This is continued till about September. During this period the young of the following fishes and prawns of economic importance, along with many less important species of estuarine fishes, enter the bheri:

FISHES

Mugilidae:
Grey mullet--Mugil parsia Hamilton, M. tade Forskal, M. corsula

Percidae:
Bhetki--Lates calcarifer (Bloch)

Siluridae:
Tengra--Mystus gulio (Hamilton)

PRAWNS

Penaeidae:
Penaeus semisulcatus De Haan
Metapenaeus monoceros Fabricius

Palaemonidae:
Leander styliferus Milne-Edwards

Recent observations have shown that by adopting suitable methods for collecting fry of various species and by selective stocking in the bheris, the production can be considerably increased.

The ecological investigations of typical bheris have shown that there are marked fluctuations in the salinity of the water during the period of culture; but the fishes and prawns growing in the bheris, being strongly euryhaline, withstand the changes and grow relatively faster. The profuse growth of benthic algae in the bheris has been found to be the main source of food for the cultivated species. Soils that have high contents of available nitrates and phosphates sustain better growths of algae. Blue-green algae grow in greater profusion in shallow waters, whereas green algae are found in larger quantities in less shallow regions. The only aquatic phanerogamic plants growing in bheris are Ruppia spp., which thrive in the deep areas. The grey mullets and the prawns feed on the algal growths in the fresh or decayed condition. The bhetki, which is a carnivore, feeds mainly on prawns and small fish. Mystus gulio is an omnivore and feeds largely on algal growths and small crustaceans.

Fishing operations start about October, when no more tidal water is drawn into the bheri. Fishing is done mainly by means of seines and traps. Mugil parsia attains a length of 10 to 15 centimeters; M. tade, 22 to 25 centimeters; and Lates calcarifer, 12 to 25 centimeters by the end of October - November. Penaeus semisulcatus reaches a length of 7.5 to 13 centimeters; Metapenaeus monoceros, 5 to 10 centimeters. Catches are generally kept in special market ponds constructed near the main sluice gates to enable the farmer to sell the fish in good condition according to demand. Many farmers transfer small fish into separate rearing ponds and release them again into the bheri during the following January or February, when fresh operations begin, to enable the fish to attain a large size by the end of the second year. The production of fish from such embanked areas during the reclamation period is estimated to be between 150 and 250 kilograms per hectare /between 133 and 223 pounds per acre/.

A rice-cum-fish culture plot. Note the canal system, which serves as a refuge for fish during the dry months.

Culture of Fish After Reclamation

Even after reclamation of the swampy land for rice cultivation, the embankment built all round the area has to be maintained in good condition, especially on the side facing the river, to protect the field from tidal flooding. The canal system and the sluice gate are also maintained in good repair to enable the drainage of excess rainwater from the fields. On an average, 8 percent of the area of such fields is occupied by canals ranging from 0.6 to 1.5 meters in depth. In many areas of the Sunderbans these canals and the adjacent ricefields are utilized for fish culture. To prevent the escape of fish from the canals when water is let in and to help in the capture of fish from the canals by means of traps when required, the mouth of the main canal leading to the field is provided with a bamboo fencing of the type commonly erected in bheris.

Harvesting of the rice crop begins in November and is over by about January, when the field, as well as the canals, is completely dry. From February or March onward, tidal water is drawn into the canals at high tides. During spring tides, when the tidal flow is strong, the young of estuarine fish present in the area gain access to the canals. Till the onset of the

monsoons, the water level in the canals is maintained at about 30 centimeters below the level of the ricefield, the depth of water varying from a few centimeters in shallow areas to about 1 meter in the deeper portions. The young fish that enter the canals have conditions favorable for their growth because of the shallowness of the water and the consequent rich growth of benthic biota.

The feeding of tidal water into the canals is stopped by about June, when the southwest monsoons begin. The fields are then prepared for the planting of rice seedlings, which is done about July. In most fields, manures such as cow dung, bone meal, ammonium sulfate, and superphosphates are applied. The rice strains cultivated in this area are salt-resistant, and the seedlings are planted at distances of 20 to 23 centimeters. By August, owing mainly to the accumulation of rainwater, the level of water in the canals rises to 0.6 to 1.5 meters, when the rice plots also get inundated. Then the embankments along the canals are cut so as to merge the water in the canals and rice plots, and this enables fish to gain access to the plots. The depth of water in the rice plots varies from a few centimeters to more than 60 centimeters. If the water level in the ricefield rises abnormally, the excess water is drained into the river, through the sluice gate, during low tides; and the bamboo fencing that protects the sluice prevents the escape of fish from the field. If required, river water is taken into the canals during the latter half of the monsoon season, when the water of the river is practically fresh (salinity being less than 0.5 part per thousand). By October or November, when the rainy season is over, the water level in the rice plots falls appreciably and, in most areas, the fields dry completely. The fish then fall back into the canal, and the fishing operations are started. All the fish from the canals are not caught at the same time. Appreciable quantities of fish are obtained every day by letting in water at high tides, when the fish swim against the tide and get caught in the traps set in the gaps of the bamboo fencing. A number of fish are caught by allowing them to enter narrow ditches along with incoming tidal water and then draining them during low tides. Finally, just before or after the harvest of rice, the canals are thoroughly netted with seines to remove all the fish.

The grey mullets and prawns feed on detritus and benthic algae in the ricefields and the bhetki on prawns and small fishes. Mystus gulio feeds on detritus, zooplanktonic organisms, small prawns, and algae. On an average, during the period of culture Mugil parsia reaches a length of 12.7 centimeters; M. tade, 33.8 centimeters; M. corsula, 17.3 centimeters; Lates calcarifer, 36.4 centimeters; and Mystus gulio, 11.4 centimeters. Penaeus semisulcatus attains a mean length of 11.7 centimeters; Palaemon carcinus, 10.5 centimeters; and Metapenaeus monoceros, 6.7 centimeters. Some of the fishes, especially the bhetki, grow much quicker in fields than in the bheris or estuaries. Any yield of 100 to 200 kilograms of fish per hectare [about 89 to 178 pounds per acre] of ricefield generally is obtained by this method of fish culture without in any way affecting the yield of rice.

The methods of fish culture either during or after reclamation of land are far from intensive, and there is much scope for improvement of production by the adoption of more intensive stocking operations and by general improvement of cultural practices. But even as it is, with practically no extra outlay a yield of 100 to 250 kilograms of fish per hectare [about 89 to 223 pounds per acre] is obtained, and the fish supplies of the State have been considerably enhanced by this.

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In press. Observations on the culture of brackishwater fishes in paddy fields in West Bengal (India). 7th meeting (1956), Proc. Indo-Pac. Fish. Coun. Bandung.

REVIEW: THE PHYSIOLOGY OF FISHES

Margaret E. Brown, editor
Vol. 1: Metabolism. 447 pp., illus. $12. Vol. 2: Behavior. 526 pp., illus. $14.
Academic Press Inc., New York. 1957.

These volumes fill a long-standing need for a compilation and summary of scattered material on fish physiology. Though comparative physiology texts have devoted space to the fishes, not since the now rare "Physiologie der Susswasserfische Mitteleuropas" by Wunder (1936) has such a complete treatment of the subject been attempted.

The first chapters of volume 1 deal with basic topics of metabolism (respiration, metabolic rate, circulation, digestion, and excretion). In an extensive section on aquatic respiration, problems in methodology and interpretation are noted and lines of future research suggested. "Active" and "standard" metabolism are clearly differentiated. The chapter on digestion offers much detail but lacks sufficient histological illustrative material. The chapter on excretion and osmoregulation contains valuable tables.

The review of the function of endocrine organs is exhaustive. In the chapter on the biochemical composition of fish, attention is given to the many sources of variability to be considered in designing tests for biochemical analysis of the whole fish or portions of it.

About a third of volume 2 deals with an analysis of behavior per se; the remainder deals with the nervous system, sense organs, and assorted topics. The best of this volume is the section on the nervous system, which offers much detail on the anatomy and function of all parts of the system. The same chapter has short sections on vision, olfaction, and hearing and equilibrium.

In the chapter entitled "Behavior," the "instinctivist" view of fish behavior is presented, the quantitative approach to behavior is upheld, and experiments on conditioned responses are discussed.

The rest of the volume consists of short, well-written chapters prepared by recognized authorities and dealing with such topics as electric and luminous organs, swim bladder, pigmentation, color changes, water quality requirements, and physiological genetics. It would be difficult, I think, to find a topic on fish physiology that has not been covered in either of these volumes.

Many comparisons with other vertebrates, particularly mammals, are made; but the emphasis throughout both volumes is--as it should be--on the unique physiological mechanisms that fish have evolved to cope with an aquatic existence. The material in this work should aid considerably in understanding the evolution of the terrestrial warm-blooded vertebrates.

This work is a "must" for all students of fish physiology and should be on the shelves of all persons interested in comparative physiology.

-- AUSTIN E. PRITCHARD, Department of Zoology, Oregon State College, Corvallis, Oregon.

EFFECTS OF CHLORETONE AND M.S. 222 ON EASTERN BROOK TROUT

Alfred L. Meister
Atlantic Sea Run Salmon Commission, University of Maine, Orono, Maine
and
Charles F. Ritzi
Maine Department of Inland Fisheries and Game, Augusta, Maine

THE CARCINOGENIC PROPERTIES OF URETHANE (Wood 1956) have prompted fishery workers to consider other anesthetics. Chloretone (chlorobutanol) (2 propanol - 1, 1, 1, trichloro - 2 methyl) and M.S. 222 (tricaine methanesulfonate) (Tricaine-Sandoz) are presently favored.

In the spring of 1957, experiments were conducted to compare these two drugs. The experiments were designed to determine practical field concentrations, to study the effects of different water temperatures, and to check anesthetized fish for subsequent (delayed) mortality.

A practical field anesthetic should produce anesthetization within approximately 5 minutes and rapid recovery upon return to fresh water. Exposure to the anesthetizing solution for an additional 10 minutes without injury was desired.

Material and Methods

Chloretone is a coarse, white, crystalline substance that sublimates readily and has a strong camphorlike odor. It is relatively insoluble in water. M.S. 222 is a fine, white, crystalline substance that is relatively stable and readily soluble in water.

Test fish were yearling eastern brook trout, Salvelinus fontinalis (Mitchill), that averaged 8.1 inches in total length (range, 5.3 to 10.3 inches).

A series of preliminary experiments was run for each anesthetic to determine the upper and lower limits of practical field concentrations at each of two temperature ranges. Several concentrations were then chosen within these ranges, and three final tests were conducted at each concentration.

Stock solutions of the two anesthetics were prepared in the laboratory using distilled water and were diluted to the desired concentration at the time of treatment. Stock solutions of 0.5 percent and 1 percent chloretone were used in the preliminary experiments. Comparison of these solutions indicated that the prolonged agitation necessary to make a 1-percent solution caused considerable sublimation of the chloretone and resulted in a solution of unknown strength. Accordingly, all final tests were conducted with a 0.5-percent stock solution. A 1-percent stock solution of M.S. 222 was used in both preliminary and final tests. Prolonged storage and exposure to sunlight may cause solutions of M.S. 222 to deteriorate; solutions subjected to these conditions tended to darken and develop a strong odor.

Six fish were used in each test, and all tests were conducted in Fernow pails containing 10 liters of solution. Fresh solutions were used for each test. Recovery was made in Fernow pails containing 10 liters of fresh water. Fish were

considered anesthetized when they lay quietly on the bottom of the test container and exhibited no movement (exclusive of respiratory movement) other than occasional flapping of the caudal peduncle and fin. Recovery was considered as accomplished when the fish were able to right themselves and maintain their equilibrium while swimming. Anesthetization time was recorded for the first, third, and last fish. Each treatment was terminated 10 minutes after anesthetization of half (3) of the test fish. Fish were then placed in fresh water, and recovery time of the first, third, and last fish was recorded.

Effects of temperature differences were tested in 2 narrow ranges: 37° to 39° F. and 48° to 51° F. Temperature of hatchery water ranged from 37° to 39° F. throughout the experiment, and it was necessary to acclimate fish to a higher temperature for tests in the 48° to 51° F. range. To accomplish this, an indoor hatchery trough was filled with fresh water and allowed to remain undisturbed until hatchery-room temperature (48° to 51° F.) was reached (approximately 48 hours). Fish were held in this warmer water for a minimum of 3 hours and a maximum of 6 hours prior to treatment. Maximum time of exposure to higher temperature before return to normal hatchery-water temperature was 6.5 hours. Treatment solution and recovery water were kept at the same temperature to which the test fish had been acclimated by partial immersion of the Fernow pails in the acclimating water.

Treated fish were fin-clipped to identify them with the anesthetic, concentration, and temperature range of the treatment; they were held in raceway pens for 10 to 40 days to permit observation of subsequent mortality.

Chloretone

The U. S. Dispensatory (Wood et al. 1933) lists the following among the general properties of chloretone: a somnifacient, a general nerve sedative and anticonvulsant, a local anesthetic causing relaxation of involuntary muscle, a preservative of pharmaceutical and biological preparations, and a bacteriostatic.

As early as 1931, Goodrich and Nichols reported using chloretone as an anesthetic for fin-clipping zebrafish (Brachydanio rerio). Those writers did not mention concentrations. Sumner and Wells (1935), working on Fundulus parvipinnis and Gillichthys mirabiles, used chloretone in a concentration of 0.05 to 0.1 of saturation at 51° to 54° F. and 90° to 95° F. Burrows (1952) used chloretone in a concentration of 1:2,500 on fingerling salmon (no species indicated). This concentration was effective in 2 to 3 minutes, with a recovery time of 3 to 8 minutes. Cope (1953) anesthetized adult cutthroat trout (Salmo clarki lewisi) at temperatures of 35° to 50° F. with chloretone in a concentration of 1:2,000. These fish required 2 to 10 minutes to recover. Nelson (1953) used chloretone in a concentration of 1:2,000 at 60.8° F. on coho salmon (Oncorhynchus kisutch), red salmon (O. nerka), and Dolly Varden trout (Salvelinus malma). He reported that no ill effects were noted if the fish remained exposed to the anesthetizing solution for a short time after being anesthetized. In the same article Nelson noted that "there was a rather small latitude of concentrations whereby mortality of the fish could be restricted to very low figures." Parkhurst and Smith (1957) used chloretone in a concentration of 1:2,500 at 43° F. on spawning adult rainbow trout (Salmo gairdneri). Fish were partly anesthetized in 1.0 to 1.5 minutes, and 96.6 percent of the fish were in good condition 75 days after spawning.

During the present experiments, preliminary tests were conducted with concentrations of 1:700 to 1:5,000 in the 37° to 39° F. range. Practical field concentrations were determined to lie between 1:1,000 and 1:3,000, and within this range five concentrations--1:1,000, 1:1,500, 1:2,000, 1:2,500, and 1:3,000--were chosen for final testing. At 48° to 51° F., preliminary tests were conducted in the range 1:1,000 to 1:5,000 and all concentrations were found suitable for field use. Final tests were conducted with 7 concentrations--1:1,000, 1:1,500, 1:2,000,

1:2,500, 1:3,000, 1:4,000, and 1:5,000--in this range. Results of these tests are plotted in figure 1.

Three hundred and forty-two fish were anesthetized with chloretone. No mortality was encountered during the anesthetization, recovery, or the subsequent observation period of 10 to 40 days.

Chloretone exhibited a marked effect on respiration. At 37° to 39° F., this influence became noticeable when concentrations were increased to 1:3,000. Fish so affected ceased normal respiratory movements and exhibited a characteristic occasional rapid opening and closing of the mouth, accompanied by a coordinated wide distension of the opercula. At a concentration of 1:3,000, these symptoms were evident after 14 to 18 minutes. Time of exposure to produce these symptoms was reduced as the strength of the solution was increased. At a concentration of 1:1,000, they were observed in 4 to 6 minutes. At a concentration of 1:700, all visible respiratory movements had ceased after exposure for 11 minutes; these fish were retained in this solution for an additional 1 minute and, when placed in fresh water, recovered fully in 20 minutes.

At 48° to 51° F., identical symptoms were observed at all the concentrations tested. They were evident at concentrations of 1:5,000 after 14 to 19 minutes and after 1.5 to 3 minutes at a concentration of 1:1,000. Respiratory movements did not cease entirely at any of the concentrations tested in this temperature range. Some convulsive body movements were observed after 5 to 10 minutes of exposure at this temperature range.

To compare the effects of chloretone crystals with the stock solutions, 10 grams of chloretone was added to 10 liters of water (a theoretical 1:1,000 concentration) and stirred for 2 minutes. Tests using this solution were conducted at each temperature range. Results indicated that the actual strength of this solution approximated 1:2,500 at both temperature ranges. Because of the relative insolubility of crystalline chloretone in water, accurate determinations of concentration are time-consuming and difficult to obtain when using this form of the drug.

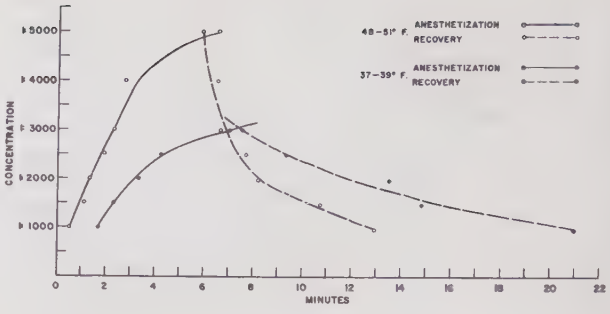

FIGURE 1.--Effect of chloretone on eastern brook trout at two temperature levels. Reported times are averages of three trials and indicate anesthetization and recovery of half of the test fish.

M.S. 222

M.S. 222 has both an antiseptic and an anesthetic action on fish and other vertebrates. It is listed as an experimental drug, and its properties cannot be found in standard pharmaceutical or chemical references. The manufacturer labels M.S. 222 containers variously as "Poisonous," "Research Material," and "To be used to calm or immobilize cold-blooded animals."

The earliest complete reference to the physiological effects of M.S. 222 was found in a series of articles by Baudin (1932-a, 1932-b, 1932-c, 1932-d, 1934), although he stated that earlier work had been done.

Baudin (1932-a), working with goldfish (Carassius auratus), found that M.S. 222 in a concentration of 1:1,000 caused "complete anesthetization" in 2 minutes but "recovery" required 30 to 60 minutes. There was a loss of muscle tonicity and a slower than normal circulation of blood in the pectoral fins. At 61° F. and a concentration of 1:20,000, "narcosis" was complete in 30 to 60 minutes and fish could live for 24 hours or longer without damage. At this concentration and temperature, an exposure of 1 hour reduced oxygen consumption to one-half of normal.

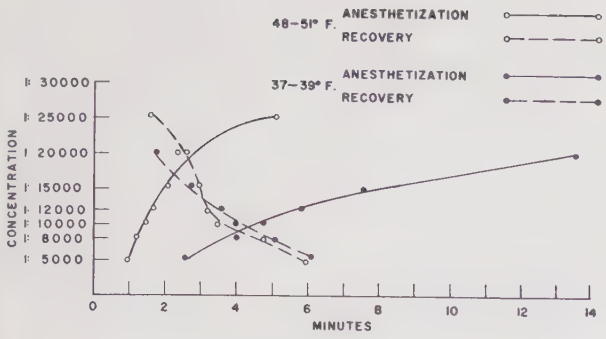

FIGURE 2.--Effect of M.S. 222 on eastern brook trout at two temperature levels. Reported times are averages of three trials and indicate anesthetization and recovery of half of the test fish.

It was reduced to one-fourth of normal with 24 hours' exposure but returned to normal 2 hours after recovery. Above 61° F., this concentration did not give complete "narcosis" nor did it slow the respiratory rate proportionately. Below 61° F., this concentration was sufficient to establish "narcosis"; but lower temperatures produced a lower rate of oxygen consumption. The most desirable concentration of M.S. 222 for anesthetization of coho and red salmon fingerlings and Dolly Varden trout was reported by Nelson (1953) as being 1:12,500 at 55° to 63° F.; however, a concentration of 1:10,000 caused 100 percent mortality. Parkhurst and Smith (1957) used a concentration of 1:3,787 at 43° F. to anesthetize spawning adult rainbow trout in 30 to 45 seconds, with some mortality when fish were left longer in this solution. Robertson (1957) used M.S. 222 in a 1:25,000 solution for anesthetization of king salmon (Oncorhynchus tshawytscha) fingerlings, 3.8 to 6.2 inches in total length, in groups of 10 or 20 fish. He also used this drug to kill the same fish at a later date but did not report the concentration used or the time required. An editorial comment accompanying an article by Wood (1956) reported that Mr. R. T. Webb, U. S. Fish and Wildlife Service, Atlanta, Georgia, used M.S. 222 at a concentration of 1:10,000 at temperatures ranging from 50° to 80° F. This concentration effectively anesthetized golden shiners (Notemigonus crysoleucas), goldfish, and bluegill (Lepomis macrochirus) but was lethal to fathead minnows (Pimephales promelas). A concentration of 1:40,000 used on bluegill reduced their activity, but the fish remained upright in a swimming position. Butler (1957) used M.S. 222 at a concentration of 1:7,570 and a temperature of 50° F. for subcutaneous tagging of large rainbow trout. Four or five fish were anesthetized at a time. As the temperature increased, it was necessary to reduce the number of fish exposed to this concentration for a given time or to reduce the strength of the anesthetic. Butler stated that fish should not be exposed to this concentration for periods in excess of 3 minutes, but that fish might be held for longer periods without harm at lower concentrations or lower temperatures. Mr. Stuart E. DeRoche, Maine Department of Inland Fisheries and Game, Augusta, Maine, reported to us that he had used M.S. 222 at a concentration of 1:12,500 on adult lake trout (Cristivomer namaycush) with excellent results. In a personal communication, Dr. Robert E. Lennon, Bureau of Sport Fisheries and Wildlife, Kearneysville, West Virginia, informed us that M.S. 222 in a concentration of 1:12,500 worked well with rainbow trout and brook trout.

At 37° to 39° F., preliminary tests were conducted with concentrations in the range 1:1,000 to 1:20,000 and final tests were conducted with concentrations of 1:5,000, 1:8,000, 1:10,000, 1:12,000, 1:15,000, and 1:20,000. Preliminary tests at 48° to 51° F. were in the range 1:5,000 to 1:30,000; and final tests at concentrations of 1:5,000, 1:8,000, 1:10,000, 1:12,000, 1:15,000, 1:20,000, and 1:25,000. Results of these tests are shown in figure 2.

Three hundred and sixty-six fish were anesthetized with M.S. 222. No mortality was encountered during anesthetization, recovery, or the subsequent observation period of 10 to 40 days.

M.S. 222 exhibited a marked effect on respiration. At 37° to 39° F., respiratory movements were regular but became progressively more labored at concentrations

increasing from 1:20,000 to 1:9,000. Distension of the opercula was observed at concentrations of 1:8,000 to 1:5,000, but respiration was still regular. After exposure for approximately 13 minutes to M.S. 222 in a concentration of 1:4,000, most fish exhibited respiratory "gasping" identical with that described for chloretone. At a concentration of 1:1,000, all respiratory movements ceased after 5 minutes in solution. Fish were retained in this solution for an additional 6 minutes and, when placed in fresh water, recovered fully in 10 minutes.

No respiratory disturbances were noted between concentrations of 1:30,000 and 1:20,000 at 48° to 51° F. Some distension of the opercula was noted at 1:15,000, and at 1:12,000 the typical respiratory "gasping" was observed in some fish after 10 to 12 minutes of exposure. All fish exhibited this "gasping" after 5 to 9 minutes at 1:10,000, after 4 to 10 minutes at 1:8,000, and 4 to 7 minutes at 1:5,000. Respiratory movements did not cease entirely at any concentration tested at this temperature range.

Discussion

Practical field concentrations of chloretone produced respiratory "gasping" in both temperature ranges. These symptoms were not evident with M.S. 222 at the lower temperatures and only at concentrations of 1:12,000 and above at the higher temperatures. Practical field concentrations of M.S. 222 interfered less with normal respiration than did those of chloretone.

The relative insolubility of chloretone makes it a time-consuming and somewhat unpredictable field anesthetic unless burdensome stock solutions are used. M.S. 222 may be used in the field as a powder, and its ready solubility ensures a known concentration.

In these experiments it appears that deep narcosis and cessation of respiratory movements cannot be considered as reliable criteria for death due to anesthetization by either drug. Fish exhibiting these symptoms for periods of 6 minutes with M.S. 222 and 1 minute with chloretone recovered fully when they were returned to fresh water. Recovery periods ranged from 10 to 20 minutes, depending on the temperature and the concentration used.

Throughout the 1957 field season, M.S. 222 was used extensively by the Maine Department of Inland Fisheries and Game. A concentration of 1:12,000 was used effectively on Atlantic salmon (Salmo salar), brook trout, and lake trout at temperatures ranging from 35° to 68° F. With young-of-the-year salmon it was necessary to reduce this concentration to prevent mortality.

The effectiveness of M.S. 222 for anesthetization of the above species can be expressed in terms of pounds of fish per gram of the drug. The following figures are those for the total pounds of fish of each species anesthetized before the drug appeared to lose its strength--necessitating the mixing of a new solution. Brook trout averaged 29 pounds per gram of drug with a range of 22 to 35 pounds. Salmon ranged from a low of 36 pounds to a high of 86 pounds per gram of drug when using spawned fish that were being jaw-tagged. Lake trout were effectively anesthetized at a rate of 93 pounds per gram of drug.

Fin-clipping operations on hatchery-reared yearling brook trout revealed an anesthetization cost of 1 mill per fish.

From field use it would appear that the effectiveness of this drug is related not only to temperature of the water but also to the physical condition of the individual fish. Sumner and Wells (1935) found that respiratory metabolism and susceptibility to anesthetics varied widely at different temperatures, in general being higher at higher temperatures. Wuhrman (1952) stated that factors affecting the action of poisons on fish are the species and age of the fish, and the pH, temperature, and oxygen content of the toxic solution. Unfamiliar combinations of these factors should be approached with due caution.

For field use, we found that M.S. 222 solutions could be reused, if desired, and were effective over a period of several days. For most field work, we found that a small amount of the anesthetic could

be prepared easily and quickly by using No. 000 gelatin capsules containing 0.8 gram of M.S. 222. When mixed with 10 liters of water, this yields a 1:12,000 concentration and has given highly satisfactory results.

Summary and Conclusions

Chloretone gave satisfactory results as an anesthetic in concentrations of 1:1,000 to 1:3,000 at 37° to 39° F. and 1:1,000 to 1:5,000 at 48° to 51° F. Recovery times were excessive at concentrations stronger than 1:2,500 in the lower temperature range and 1:2,000 in the higher. Practical field concentrations encompassed a narrow range: 1:2,500 to 1:3,000 at 37° to 39° F. and 1:2,000 to 1:5,000 at 48° to 51° F.

M.S. 222 gave excellent times as an anesthetic in concentrations of 1:5,000 to 1:15,000 at 37° to 39° F. and 1:5,000 to 1:25,000 at 48° to 51° F. Recovery times were excellent throughout these ranges, and all concentrations were suitable for field use.

A more rapid anesthetization was exhibited by both drugs with an increase in temperature. Recovery times were also more rapid, but this may have been the result of a shorter total exposure time.

The results of these experiments indicate that because of a wider range of practical field concentrations, apparent lesser inhibitory effect on respiration, and ease and predictability for use in the field, M.S. 222 is superior to chloretone as an anesthetic for fishery use on eastern brook trout.

Comparisons of M.S. 222 and chloretone presented here are based on one species at two temperature ranges. The data from this experiment may not apply to other species or at higher or lower temperatures.

Acknowledgments

Particular thanks are due Mr. G. K. White, Superintendent, Craig Brook Fish-Cultural Station, Bureau of Sport Fisheries and Wildlife, East Orland, Maine, for facilities and assistance with field work. Special thanks are due Dr. W. H. Everhart and the Maine Department of Inland Fisheries and Game for equipment, transportation, and valuable assistance. Thanks are also due Dr. R. B. Hill and the Department of Zoology, University of Maine, for laboratory facilities and constructive criticism. The M.S. 222 used in this experiment was donated by Sandoz Pharmaceuticals, Hanover, New Jersey.

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VITALITY AND SURVIVAL OF RAINBOW TROUT FED THE STANDARD DIET AND THE PELLET DIET

From May 1957 to May 1958, experiments were run under controlled stream, pond, and starvation conditions to compare the vitality and survival of catchable-size rainbow trout that were received from a California hatchery, divided into two equal groups, and then fed the standard all-meat diet and the pellet diet.

Test and number of days		Standard diet				Pellet diet			
		Mean length (cm.)	Mean weight (gm.)	Mean condition (K)	Survival (%)	Mean length (cm.)	Mean weight (gm.)	Mean condition (K)	Survival (%)
As received	0	22.6	120.8	1.040	----	22.1	103.5	0.954	----
Stream	64	23.1	108.8	0.869	79.8	22.9	99.8	0.826	93.6
Do.	345	23.1	90.0	0.732	52.8	23.1	90.0	0.732	33.0
Pond	345	22.9	87.7	0.714	34.0	23.1	86.5	0.708	24.0
Starvation	232	22.6	66.5	0.645	16.0	22.1	63.7	0.586	16.0

The table is a summary of the length, weight, condition, and survival data on the two groups of trout.

--REED S. NIELSON, Bureau of Sport Fisheries and Wildlife, Convict Creek Experiment Station, Bishop, California.

A POSSIBLE CAUSE OF "SUNBURN" IN FISH

D. C. DeLong, J. E. Halver, and W. T. Yasutake
Bureau of Sport Fisheries and Wildlife
Western Fish Nutrition Laboratory, Cook, Washington

A LESION DESCRIBED AS A GRAY PATCH GENERALLY LOCATED ANTERIOR TO THE DORSAL FIN has been associated with high mortality of fish on numerous occasions in production hatcheries throughout the United States. This lesion has been called "sunburn" or "backpeel." No bacteria or other pathogens have been found in fish with these symptoms. For example, at a Montana hatchery in April 1956, mortality of 10 to 15 percent occurred daily and this lesion was the only syndrome found.[1/] On occasion, shade has prevented this condition and even restored affected fish to an apparent normal condition; thus there has seemed to be a correlation between sunshine and the condition in fish. To our knowledge, this has been the only therapy attempted.

In man there is a similar condition associated with pellagra, a disease that is produced in general by a niacin deficiency. The lesion in man, found almost exclusively on exposed areas of skin, is described as a dermatitis irritated by sunlight. The effect of sunlight on pellagra was studied extensively by Smith and Ruffin (1937). Those workers showed conclusively that sun rays precipitated acute manifestations of pellagra when susceptible subjects, who had been subsisting on a deficient diet, were exposed to sunlight. Exposing only a particular area of skin to induce the lesions at a particular site is a procedure that has been used successfully with man. Smith and Ruffin (1937) found that keeping patients with pellagra in dark rooms or improving their diets aided in their recovery, with a combination of these measures providing the best therapy. The incidence of this disease in man increases to a maximum during the spring and summer when there is more sunshine.

It has been suggested that a similar clinical sumptom may occur when phenothiazine is ingested by fish and the population exposed to direct sunlight (Rucker, in press). Therefore, in any feeding test with fish, careful control of dietary ingredients must be considered.

The similarities between the lesions of fish and of man were noted with interest. The fact that no causative agent could be found in these fish indicated the possibility of a nutritional deficiency. An experiment was conducted to determine whether a niacin deficiency in fish caused a susceptibility to irritation by sunlight.

Experimental Work

The diet used in this experiment was that of Halver and Coates (1957), with the following modifications. The casein and gelatin components were reduced proportionately to lower the protein of the diet from 70 percent to 50 percent. The

[1/] Personal communication from Dr. R. R. Rucker, Chief, Western Fish Disease Laboratory, U. S. Fish and Wildlife Service, Fishery Center, University of Washington, Seattle 5, Washington.

Mr. DeLong's present address is: Department of Biochemistry, Purdue University, West Lafayette, Indiana.

reduction in protein was made on the basis of the requirement at this temperature (DeLong, Halver, and Mertz, 1957). The weight of all deletions from the original diet was replaced by dextrin, and the L-methionine and L-tryptophan supplements were eliminated.[2] Tryptophan is a known precursor of niacin (Krehl et al. 1945) which could modify the niacin deficiency (Horwith et al. 1956). In the niacin-deficient diet, niacin was absent from the crystalline vitamin mix and the recovery diet had the same niacin content as the control diet; i.e., 80 milligrams per 100 grams of dry weight.

For the feeding trial, 200 chinook salmon averaging 3.17 grams each were hand-counted into an experimental hatchery trough, 10 by 12 by 48 inches. The trough was not covered and was placed where the fish received the maximum natural sunlight at the experimental hatchery facilities at Hagerman, Idaho. Daily mortalities were recorded. The fish were fed the niacin-deficient diet for 30 days. After 30 days the population was split into two equal groups, one remaining on the niacin-deficient diet and the other group receiving the complete diet. Histological samples were taken at the end of each 30-day period, fixed in Bouin's solution for 24 hours, and stored in 65 percent alcohol until used.

Results

Eighteen days after the feeding of the niacin-deficient diet had started, lesions similar to those of "sunburn" were observed. These lesions resembled, both grossly and histologically, those seen in production hatcheries.[3] The first mortality was recorded on the 22d day. In the next 8 days, 22 fish died, all having severe lesions. Fungus had started to grow at the site of the lesions in some of the fish, indicating that the cells had died. Fourteen days after the fish were divided, 15 of 83 fish receiving the niacin-deficient diet had died and 5 of 82 receiving the recovery diet had died. These were the last mortalities in either lot. Beginning within a few days after the fish were divided, cloudy weather prevailed until the end of the experiment.

Discussion

The data presented in this report are considered preliminary. Our study was hampered by cloudy weather which may have allowed the fish receiving the niacin-deficient diet to overcome the acute manifestations. The difference in mortality in the two subgroups is indicative, however, of a possible response to dietary therapy.

The data are presented now because the real test of our hypothesis would be to have a production hatchery confronted with this problem treat its fish by supplementing the diet with niacin. Necessary precautions should be taken to insure proper mixing of the diet if crystalline niacin is used, and methods of feeding should provide maximum availability of the niacin to the fish.

Attention should be called to the fact that though this lesion is the visual symptom, there are other serious effects from a niacin deficiency as described previously (Halver 1957). These other effects could still be present after recovery from the "sunburn" lesion through shading from the sun, but could be eliminated by use of a dietary supplement. As the metabolism of phenothiazine may result in an antimetabolite to niacin, exposure to direct sunlight of fish receiving the compound could result in clinical symptoms similar to the symptoms occurring with niacin deficiency.

The study of this type of dermatitis in man has been hampered by the lack of an experimental animal that responds similarly. Perhaps fish could be the experimental animal that would allow this study to proceed.

[2] Unpublished recent data indicate that these supplements are not needed at the 50-percent protein level.

[3] Additional histological data will be presented after this experiment has been repeated.

Summary

Attention is drawn to the similarities of a dermatitis irritated by sunlight in man and in fish. Preliminary data are given, these suggesting that, as in man, the photosensitivity in fish is related to a niacin deficiency.

Acknowledgment

The authors wish to thank R. R. Smith and M. E. Larson for the care and feeding of the experimental fish.

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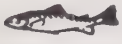

IN-SERVICE TRAINING FOR FISH-CULTURISTS

In-service training for fish-culturists seems to have begun with a short course presented in 1936 at Montana State College by Dr. C. J. D. Brown, then instructor and now professor at that institution. This intensive 6-day course covered identification of fish-food organisms, examination of scales and otoliths for age determination, and methods of food-habits study through stomach analyses. Fifteen students--employees of the State of Montana, the U. S. Forest Service, and the U. S. Fish and Wildlife Service (then the Bureau of Fisheries)--completed the course.

Just before World War II, trainees from all parts of the country were brought to the Leetown, West Virginia, laboratory and fish-cultural station for a 3-month course presented by Mr. Eugene Surber, Dr. H. S. Davis, and others. Outbreak of war forced discontinuance of this school.

The Bureau of Sport Fisheries and Wildlife now has four in-service training schools for its fish-culturists: at Cortland, New York (nutritional problems in trout culture); at Leetown, West Virginia (fish diseases); at Marion, Alabama (culture of warm-water fishes); and at Seattle, Washington (culture and diseases of salmonids other than trout). The total enrollment in the 1958-59 training year will be 17. Of these trainees, 14 are Federal employees; 3 are employees of the State fishery agencies of New York, Oregon, and Virginia.

THE ORGANIC COMPOSITION OF BROOK AND BROWN TROUT BLOOD

Arthur M. Phillips, Jr.
Bureau of Sport Fisheries and Wildlife
Cortland, New York

A KNOWLEDGE OF THE CHEMICAL COMPOSITION OF THE BLOOD OF HIGHER ANIMALS has provided a means for diagnosis and treatment of many of their pathological conditions and a basis for the formulation of synthetic media for culturing and studying the disease organisms. The information about the composition of the blood of trout is meager. Field, Elvehjem, and Juday (1943) reported the inorganic composition of carp blood and some of the organic components of trout blood. Phillips and his coworkers (1957) determined the composition of the blood of brown trout for 11 of the inorganic components. In an effort to provide a more complete picture of the composition of trout blood, analyses for the organic components of both brook and brown trout blood were completed during the summer of 1957 at the Cortland laboratory.

Method

Samples of blood were withdrawn from 2-year-old brook and brown trout. The trout were large enough to assure adequate blood samples but too young to spawn during the year. Avoidance of spawning was considered important to preclude abnormal blood composition that might be caused by sexual activity. The brown trout averaged approximately 300 grams in weight and the brook trout approximately 350 grams. To assure standard conditions at the time of blood sampling, the trout were fed at 4 o'clock in the afternoon of the day preceding their experimental use and the samples of blood were withdrawn the next morning at 7 o'clock before the fish were fed. This practice avoided confounding changes in blood composition that might occur if digestion and absorption of food were taking place at the time the blood was sampled.

The blood was withdrawn from the fish by cardiac puncture with a 3-cc syringe equipped with a 1.5-inch No. 26 hypodermic needle. The size of the blood sample taken was dependent on the analysis under consideration and ranged from 1 to 15 cubic centimeters. It was necessary to pool samples of blood from several fish to obtain the larger samples of blood. A particular fish was sampled only once during the experimental period.

Phillips and his coworkers (1957) reported that occurrence of some hemolysis was evidenced by a pink color of the blood serum. Only the analyses for serum iron were affected by hemolysis in these earlier studies. In the present experiments such hemolysis could introduce a large error in the analyses of the protein fractions of the blood. Centrifuging the blood immediately after the sample was taken prevented hemolysis.

Analyses were made (dependent on the blood component under study) from whole blood, blood serum, and blood plasma. The table lists the components studied and the portions of the blood on which the analyses were made. All analyses were made in accordance with the methods

Component	Whole blood		Blood serum		Blood plasma	
	Brook trout	Brown trout	Brook trout	Brown trout	Brook trout	Brown trout
	Milligrams per 100 cubic centimeters					
Lactic acid	10.2	12.6	------	------	------	------
Cholesterol	247	287	316	402	------	------
Glucose	70	71	------	------	------	------
Cholesterol ester	84	95	61	67	------	------
Total nitrogen	------	------	368	347	422	417
Nonprotein nitrogen	------	------	24.6	25.0	------	------
Uric acid	------	------	.87	.90	------	------
Urea	2.9	2.8	2.6	2.6	------	------
Creatine	1.21	1.01	.69	.61	------	------
Creatinine	.41	.46	.41	.49	------	------
Amino acid	17.70	18.70	16.90	17.71	------	------
	Grams per 100 cubic centimeters					
Total protein	------	------	2.01	2.04	2.47	2.29
Albumin	------	------	1.38	.89	------	------
Globulin	------	------	.63	1.15	------	------
Hemoglobulin	10.2	8.8	------	------	------	------
Fibrinogen	------	------	------	------	.44	.44

recommended for use with the Lumetron Photoelectric Colorimeter.[1/]

The results of the blood analyses are shown in the table. The values in the table represent the averages of the analyses of four samples of blood, each determined in duplicate. Variation of blood composition from day to day was taken into consideration by using no more than one sample of blood per day for the analyses of any one component of the blood.

Discussion

In general, where comparable, values for the organic components of brook and brown trout blood in these studies approximate the values reported for rainbow trout by Field, Elvehjem, and Juday (1943).

In only two instances are there differences in the composition of the blood of brook trout and brown trout. First, the cholesterol is higher in the whole blood and the blood serum of brown trout than in the comparable fractions of brook trout blood. Second, the albumin is higher and the globulin lower in the serum of brook trout blood than in the serum of brown trout blood. In the blood of higher animals, the ratio ranges between 2 and 3 parts of albumin to 1 part of globulin. A ratio of 2 parts of albumin to 1 part of globulin occurs in the brook trout blood serum in this study and in the blood plasma of rainbow trout as reported by Field, Elvehjem, and Juday (1943). A reversed ratio of albumin to globulin occurs in the blood of the brown trout used in this study. Both an increase in blood cholesterol and a reversal of the ratio between albumin and globulin are characteristic of nephritis and nephrosis of kidney tissues of higher animals (Hawk and Bergeim 1937). Microscopic examinations of the kidney tissues of the trout used in this study revealed an abundance of the diplobacillus

[1/] Manual for the Lumetron Photoelectric Colorimeter. Photovolt Corporation, New York 16, New York. (Mimeographed)

organisms associated with kidney disease in the brown trout, but only an occasional pair of these organisms was found in the kidney tissues of the brook trout. Though the brown trout used in these studies have been under observation for several months since completion of the blood analyses, active lesions associated with kidney disease of trout have not appeared. It is possible that the brown trout used in these experiments are harboring a latent case of kidney disease. If this correlation is substantiated in future studies of blood composition and trout kidney disease, a real advance in fish pathology will result. The value of such correlations in diagnosing fish disease and in judging the efficiency of disease treatment is great.

The cause of the rapid coagulation of fish blood is of great interest to physiologists. The fibrinogen content of brook and brown trout blood (0.44 gram per 100 cubic centimeters of blood) lies intermediate in the range of the fibrinogen content of human blood (0.3 to 0.6 gram per 100 cubic centimeters of blood, Hawk and Bergeim 1937) and is twice the level reported by Field, Elvehjem, and Juday (1943) for rainbow trout (0.22 gram per 100 cubic centimeters of blood). Thus the fibrinogen content of fish blood cannot account for its rapid coagulation, a conclusion also reached by Field, Elvehjem, and Juday (1943).

These values (see table) for the organic components of brook and brown trout blood and its fractions should serve as a starting point in the establishment of normal values for these species of trout. After normal values are firmly established, a comparison of these values with values obtained from the fish under pathological conditions should be undertaken. In this manner a diagnostic table for various fish diseases, based upon blood composition, can be established.

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EXPERIMENTAL FEEDING OF A PELLETED TROUT FOOD TO LARGE FINGERLING AND YEARLING BROOK, BROWN, AND RAINBOW TROUT, 1955-1956

by Robert E. Schumacher, Department of Conservation, St. Paul, Minnesota
--Volume 20, number 2, pages 51-57. April 1958.--

CORRECTIONS

On page 55, table 6: under the heading "Mean hemoglobin," the first column should be corrected to read Grams per 100 milliliters instead of Grams per liter; under the heading "Mean red blood cells," the first column should be corrected to read Red blood cells per cubic mm. instead of Red blood cells per cubic cm.

SELECTIVE POISONING AS A MANAGEMENT TOOL IN STRATIFIED TROUT PONDS IN MASSACHUSETTS

William A. Tompkins and James W. Mullan
Massachusetts Division of Fisheries and Game
Westboro, Massachusetts

USE OF ROTENONE AS A FISHERY-MANAGEMENT TOOL has increased tremendously during the past several years. The popularity of this chemical is a direct result of its acceptance by fishery administrators, biologists, and sportsmen.

Correlated with its use for eradicating entire fish complexes has been its utilization in the partial control of undesirable fish populations. In truth, many so-called complete reclamations were, and are still, partial eradications. Various authors have theorized that incomplete kills are the result of springs and seepage, loss of toxic effect in cold water, incomplete coverage, thermal barriers, or a combination of two or more of these factors. The first reported observation and the first suggestion that fishery management might benefit from planned selective poisoning were put forth (Greenbank 1941) as a result of the rotenoning of two small trout ponds in Michigan. The author felt that the application of low concentrations of rotenone to the epilimnion would be a means of controlling undesirable fish in certain trout waters. This project was not followed up, however, and most of the work on rotenone to date has been aimed at devising equipment and methods to provide for the complete dispersion of the chemical throughout the total volume of any given lake or pond. Clemens and Martin (1953), in a study of rotenone dispersion in Oklahoma farm ponds, found that 5-percent emulsifiable rotenone acted in the same manner as the powdered product used in former experiments. Even in small, shallow stratified ponds, surface spraying with concentrations of as much as 2 p. p. m. did not result in attainment of biologically measurable amounts in the deeper waters. The authors concluded that the lack of penetration was possibly due to "an inactivation of the rotenone by chemical action in the lower depths and/or changes in density with increase of depth and lack of agitation of the bottom waters."

During July 1955, a severe fish kill in Big Alum Pond in Sturbridge, Massachusetts, was reported. Field investigation established that the kill had been fairly severe and largely restricted to smallmouth bass and sunfish. The cause of the mortality was obscure, but it presented an opportunity to thin overabundant yellow perch and sunfish, inasmuch as the fishing public felt that the bass had been completely wiped out by the fish kill.

Big Alum Pond has a surface area of 195 acres, a maximum depth of 45 feet, an average depth of 23 feet, and--at the time of the poisoning--a sharply defined thermocline at a depth of 10 feet. Trout-supporting water was restricted to that volume lying between the 14-foot contour and the 19-foot contour. Four months before the poisoning, this pond had received 4, 300 brown trout (9 to 12 inches in length). Forty acres of the pond, including all shoal areas and 25 acres of open water, was treated on July 29 with enough 5-percent emulsifiable rotenone to give a concentration of 0. 5 p. p. m, down to the thermocline. In the following 4 days, a little more than 5, 000 pounds of fish was

recovered. The majority of these fish were stunted yellow perch and sunfish. One trout was killed during the partial reclamation. On the basis of the known standing crops of similar population complexes in the area, it was estimated that approximately one-third of the poundage of fish in the pond was eradicated. The trout population in the colder areas was not affected by the operation. Good catches were reported only a few days later and until the end of the season.

In view of the published reports available and the results of the poisoning of Big Alum Pond, it was decided to expand the project into an attempt to treat the entire epilimnion of another trout pond. The present paper deals with the selective poisoning of Cliff Pond, Brewster.

Description and History

Cliff Pond, Brewster (Barnstable County, Cape Cod), has a surface area of 193 acres and maximum and average depths of 88 and 32 feet, respectively. The pond lies at an elevation of 26 feet and is completely landlocked. Stratification occurs in the summer, and a 30-foot layer of trout water is usually present. Late in the summer, the top of this layer is at a depth of 30 to 35 feet. The trout zone constitutes about 27 percent of the total water volume, and 42 percent of the pond bottom is exposed to it. Water transparency is 23 feet. Methyl orange alkalinity in August runs from 15 p.p.m. at the surface to 5.0 p.p.m. at the bottom. The pH ranges vertically from 6.9 to 5.5. Sand, gravel, and rubble predominate as bottom types. The pond is readily accessible, being in the Nickerson State Forest, and is surrounded by camping sites that are usually filled to capacity from June to September and to a lesser degree from April to October. Boats and motors are allowed, and a boat livery is maintained.

Prior to its reclamation, the pond contained established populations of smallmouth bass, chain pickerel, brown bullhead, yellow perch, pumpkinseed, sunfish, killifish, bridled shiner, smelt, and a few large landlocked eel.

Trout are maintained through stocking. State stocking records show the following: (1937-49) 2,465 brook trout, 6 to 14 inches in length; (1932-56) 40,000 rainbow trout, 6 to 12 inches; and (1941-56) 13,700 brown trout, 6 to 12 inches.

From 1949 to 1954, an intensive creel census was operated on the pond (Mullan 1955). During this period the pond was stocked annually with an average of 6 pounds per acre of 9- to 12-inch brown and rainbow trout. Annual trout harvest over this period came to 3.6 pounds per acre. During the census period, the harvest of warm-water fish averaged 3.2 pounds per acre per year, of which yellow perch and brown bullhead formed 87 percent. An annual average of 18 angler trips per acre was recorded. The relatively low fisherman success (0.12 fish per hour) was a reflection of two factors. Most fishermen were reluctant to keep the smaller pan fish, and many of the anglers were seeking trophy-size carryover trout. The average carryover trout taken during this period weighed 2.9 pounds, and carryovers made up 54 percent of the total weight of trout harvested. The population of landlocked smelt is believed to have been the major factor accounting for the rapid growth and survival of the trout stocked.

Creel-census data indicated that the smallmouth bass population was declining. During the entire 6 years of the census, only 276 smallmouth bass were taken. Of these, 111 were checked in 1949.

During the census period the size of the yellow perch taken decreased from an average of 0.40 pound in 1949 to 0.28 pound in 1954. These figures are not, however, representative of the total catch, as fishermen were prone to throw back all but the larger individuals. By 1955, 4- to 8-inch yellow perch could be caught literally by the hundreds. Most trout fishing at Cliff Pond is done with live bait, principally shrimp or worms; and as the summer progresses, the bait must be fished at depths below the thermocline. The problem of getting the bait down through the layer inhabited by yellow perch became a challenge in itself. Experienced

fishermen used heavy sinkers to drop the bait as rapidly as possible. This method was combined sometimes with an alternate method of chumming on one side of the boat and--when, in the opinion of the fisherman, any perch in the vicinity had been lured to that area--frantically throwing out the hooked bait on the opposite side of the boat. To the casual or uninitiated fisherman (a category including the great majority of the summer campers), the nuisance value of the yellow perch was of much greater magnitude.

Results of Poisoning

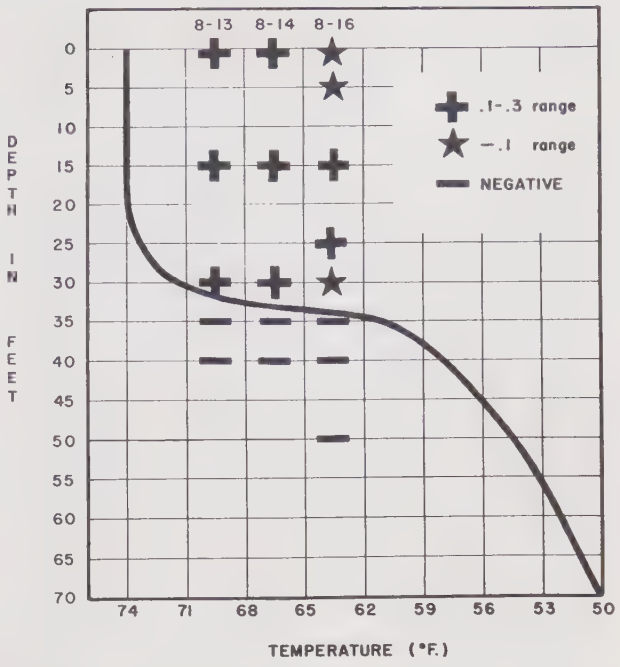
Three hundred gallons of 5-percent emulsifiable rotenone was applied to the surface of Big Cliff Pond on August 13, 1956. This amount was calculated to give a concentration of 0.2 p.p.m. of the carrier down to a depth of 30 feet. The graph shows the vertical temperature distribution of the pond when the rotenone was applied.

Application was made with two spray units working over previously plotted courses. Every effort was made to assure as even a distribution as possible.

Yellow perch began to appear on the surface while the spraying was in progress (see table) and formed the bulk of the first day's pickup. On the second day, brown bullhead became very much in evidence but yellow perch again constituted the greater bulk. A day later, recently dead and dying bullhead made up the larger percentage of the fish recovered; at this time, the pickup of yellow perch was restricted, with few exceptions, to those killed earlier but not recovered immediately.

On the first day of poisoning, 1 brown trout, weighing 0.5 pound, was recovered from the surface soon after the spraying was begun. No other trout were seen or reported during the 5 days of intensive pickup, and none were seen or reported by creel-census agents who checked the shores of the pond sporadically during the following week.

Of the approximately 51,000 yellow perch (exclusive of young-of-the-year)



The vertical temperature distribution of Cliff Pond, Brewster, at the time of rotenone application, and the results of chemical tests taken 4 hours, 24 hours, and 72 hours later.

collected during the poisoning, the mean and modal average length was 5.9 inches. Few yellow perch longer than 9 inches were taken in the poisoning, and none longer than 12 inches. Scale examinations indicated that these fish were comparatively slow-growing. The great majority of the yellow perch failed to reach a length of 6 inches prior to their fifth year.

Chain pickerel and smallmouth bass formed only 1 percent of the total kill, and young-of-the-year of both species were insignificant enough to be classified as rare.

Post-Poisoning Tests

Four hours after application of rotenone was completed, chemical sampling of the water in Cliff Pond was undertaken. The chemical test procedure followed that used by the Wyoming Game and Fish Research Laboratory (Post 1955) and utilized the color charts developed by the latter. Control samples were run from Little Cliff

Pond. This body of water is separated from Cliff Pond by a sandy bar approximately 50 yards wide.

Samples taken at the surface, at 15 feet, and at 30 feet gave positive results in an estimated range of 0.1 to 0.3 p.p.m. (see graph). It should be pointed out that these estimates were based on laboratory tests made on a large series of known values by the technician doing the field analysis. Samples collected at 35 feet and 40 feet were definitely negative and could not be distinguished from the control samples.

Twenty-four hours after the application the same series of samples was run again, and the results were identical. The second set of samples was taken in a different sector of the pond.

Seventy-two hours after the poisoning the third and last series was taken. Between this series and the former the lake had been subjected to relatively high winds reaching gale velocities, and it was considered quite likely that the circulating currents and any resulting turbulent eddies might have caused the rotenone to penetrate the thermocline. Samples taken at the surface and at a depth of 5 feet showed only a trace of rotenone, estimated to represent a concentration of less than 0.1 p.p.m. Samples at 15 feet and 25 feet showed a rotenone concentration of 0.1 to 0.3 p.p.m. The sample taken at 30 feet proved colorimetrically similar to the surface samples. Negative tests were obtained at 35, 40, and 50 feet. The decrease in concentration of rotenone at the surface and at the bottom of the epilimnion was quite probably associated with the circulating currents established by the previously mentioned strong winds and by the presence of a relatively thick area of slower-moving water above and below the shearing plane. The authors are not aware of, nor do they wish to postulate further on, the physical and/or chemical reasons for the dissipation pattern displayed in this instance.

Three 100-yard gill nets, made of mesh graded from 1.5 to 5 inches stretch measure, were fished for 48 hours in the pond. These sets were made on the evening of the 15th and completed on the evening of the 17th. During the first 24-hour period 2 nets were fished in water 40 to 60 feet deep and the other in water 10 to 30 feet deep. During the second 24-hour period 2 nets were shifted into the shallow water and the other into the deeper area.

No fish were taken in those sets made above 30 feet. In the deep nets 13 brown trout averaging 17 inches and 6 yellow perch between 12 and 15 inches in total length were taken. Fresh smelt were found in the stomachs of 6 of the trout examined; all of the perch contained crayfish or crayfish remains.

Forty-eight hours after the poison was applied, and while the pickup was going

SUMMARY OF FISH RECOVERED, IN POUNDS, DURING THE PARTIAL POISONING OF CLIFF POND, BREWSTER

Species	August					Total
	13	14	15	16	17	
Smallmouth bass-----	17.5	15.0	10.0	1.0	-----	43.5
Chain pickerel------	16.4	14.0	10.0	-----	1.0	41.4
Yellow perch--------	970.2	2,687.0	591.8	57.2	22.0	4,328.2
Brown bullhead------	10.3	1,500.0	1,114.8	240.8	87.0	2,952.9
Mummichog----------	-------	-------	-------	-----	-----	Trace
Smelt---------------	-------	-------	-------	-----	-----	Trace
Killifish----------	10.2	688.0	171.8	1.0	1.0	872.0
Bridled shiner------	3.0	5.0	4.0	-----	-----	12.0
Eel-----------------	-------	5.0	-------	7.0	-----	12.0
Brown trout---------	.5	-------	-------	-----	-----	.5
Total----------	1,028.1	4,914.0	1,902.4	307.0	111.0	8,262.5

on, an angler took a 3.5-pound brown trout while fishing at a depth of 35 feet. Trout weighing as much as 6.5 pounds were taken from the pond before the close of the season on October 20. Scattered reports of catches of "jumbo" perch were received by Division personnel during this period.

Within a 30-mile radius of Cliff Pond, 8 landlocked ponds totalling 241 acres have been reclaimed. Gross similarities in standing crops, species complexes, and physical - chemical relationships of these ponds suggest that the warm-water fish population in Cliff Pond would not differ significantly. These ponds had standing crops of 40 to 60 pounds per acre. The total fish kill at Cliff Pond was 42.9 pounds per acre. It is believed that this represents a removal of 70 to 85 percent of the total standing crop, inclusive of trout. The greater part of the weight of fish left in the pond is probably composed of smelt; the bulk of the remainder is probably composed of trout, yellow perch, and bullhead. It is not believed, however, that any species was completely eradicated.

The fact that 1.5 tons of brown bullhead was killed during the operation substantiated the authors' belief that the conclusion reached by Burdick, Dean, and Harris (1955) relative to the toxicity of rotenone to the brown bullhead is open to question. They concluded, on the basis of laboratory tests made in 60° F. water, that a dosage of 0.5 p.p.m. was inadequate if a complete kill of any fish populations which included bullhead was required. Bullhead populations have been completely removed from Massachusetts ponds with concentrations of 0.5 p.p.m., but the magnitude of the kill at 0.2 p.p.m. was somewhat surprising.

The survival of yellow perch was not unexpected, as many total reclamations in Massachusetts had shown that deep spraying was necessary in order to bring up all of the yellow perch in the ponds in question. It is interesting to note that no yellow perch of more than 12 inches in length were taken in the poisoning, and no yellow perch of less than 12 inches were taken in the experimental netting. Relationships between size and midsummer depth distribution have been noted in this species and in others, both by technicians and by fishermen, but hardly to the degree illustrated here.

Previous partial poisonings applied to trout lakes have had as their objective the eradication or suppression of coarse fish that were detrimental to trout fishing. So-called "two story" pond management has been practiced in Massachusetts for some 25 years. This terminology, currently in vogue among fishery biologists, actually implies nothing more than the stocking of catchable trout in lakes and ponds that contain warm-water species. In a few instances this has been combined with netting or shoreline poisonings of coarse fish. No attempt has been made, however, to develop more fishable populations of warm-water species that would complement the trout fishing already in existence. Partial poisonings of warm-water ponds in Massachusetts in which 40 to 80 percent of the expected standing crop was removed have shown good results, even in situations where a dozen or more species of fish were present. The immediate result of a partial fish kill of any magnitude is an increase in the growth rates of all species in the pond. The increments of this increase depend on the volume of the particular niches opened to the various species. There is no doubt that, as time goes on, interspecific and intraspecific competition will bring the fish populations back to the static balance present before the reduction. This regression can be slowed by the establishment, or reestablishment, of a strong predator population, a condition more easily brought about when the predator species can avail itself of portions of the habitat previously shared with the species suppressed by the poisoning. In some instances this is accomplished by the increased reproductive success of the remaining predators and better survival of the fingerlings, as evidenced in several warm-water ponds here in Massachusetts. Similar results are attained in newly flooded impoundments. In most partial poisonings in Massachusetts, fingerling

predators are added. Cliff Pond will be stocked with fingerling smallmouth bass in the spring of 1957. How long the smallmouth population will maintain itself is not known. Division of Fisheries and Game records indicate that this species was originally introduced in 1928. A stocking of 10,000 fingerling smallmouth produced good to excellent fishing for about 15 years. No other smallmouth were stocked after the 1928 introduction. By the middle 1940's sportsmen's reports indicated a decline in the fishing for this species, and these reports were substantiated by the creel-census data and the results of the reclamation.

The presence of a totally unknown, but probably sufficient, number of brood-stock yellow perch will produce what should be (based on other reclamation work in Massachusetts) strong year classes in 1957 and 1958--classes that will remain dominant in the fishery for at least 6 years. The first year class of yellow perch spawned after total or partial reclamations are generally fast-growing fish. Age and growth data on the fish of 5 partially or totally reclaimed waters comprising 236 acres were collected during 1956. These ponds had produced their first post-poisoning year class of yellow perch in the spring of 1954. At the time of the formation of the second annulus on these fish, their average total lengths in the 5 ponds were 7.0, 7.7, 7.7, 9.0, and 9.2 inches. When these ponds were reclaimed, the average total length of the yellow perch, excluding young-of-the-year, was 5.7 inches. The average second-year yellow perch in Massachusetts waters has a total length of 5.4 inches (Stroud 1955). Analysis of the stomachs of the 2-year-old perch from reclaimed ponds showed them to be feeding heavily on yellow perch young-of-the-year. This predation complements that of the chain pickerel and/or bass also present in these ponds and has a pronounced effect on the survival rate of later yellow perch year classes.

In the fall of 1956, after reclamation, 10,000 yearling rainbow trout and 2,000 yearling brown trout were stocked in Cliff Pond. It is probably true that yellow perch, smallmouth bass, and trout occupy the same trophic level. Superficially, it would not appear reasonable to desire all three species in the same pond. It should be pointed out, however, that trout, yellow perch, and smallmouth bass are all relatively omnivorous, and their food habits are based on availability. During a part of the year, the influence of habitat segregation will reduce interspecific competition. And, as previously pointed out, yellow perch cannibalism will help to suppress rapid numerical expansion of this species until the first year classes have been depleted. During the first few years after poisoning, the trout fishery will undoubtedly benefit by the reduction of the warm-water population. Hayes and Livingstone (1955) reported that the trout harvest from a 54-acre lake in Nova Scotia was doubled through partial poisonings of the shoal areas for 5 consecutive years. The total weight removed per acre, however, was only 6 pounds for the 5 years the pond was treated. According to their hypothesis that the increase in trout harvest was due to the removal of chubs and yellow perch only (which amounted to 1.2 pounds per acre), and considering that 25 pounds of yellow perch alone per acre was removed from Cliff Pond, the increase in trout harvest should be of much greater magnitude during the immediate post-poisoning years. Hayes and Livingstone, however, came to the conclusion that partial poisoning is as efficient as complete reclamations in establishing maximum trout production. The present writers believe that selective poisoning can be a useful tool in producing more fishable warm-water populations in ponds capable of supporting both trout and warm-water fish. They do not believe, however, that if maximum trout production is to be attained, the presence of any other species is desirable, regardless of that species' trophic level.

Thus the effects of partial poisonings of this nature are not expected to ensure that the pond in question will not revert to its pre-poisoning status several years later. Fresh-water fishery management is based on the development of artificial

population structures. No such populations, including those in farm ponds, have yet been shown to maintain the desired "balance" without the application of maintenance management. In this instance, maintenance will be the reapplication of rotenone at the intervals dictated by the rapidity with which the population assumes its pre-poisoning structure.

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EASY-TO-OPERATE GILL NETS

When fishing nylon gill nets experimentally in small gangs from outboard motorboats, our most frustrating problem has been tangling, caused by leads and floats falling through the webbing.

Trying to overcome these difficulties, we had our last nets built to the following specifications: Depth, 8 feet; length, 150 feet; selvage, double; thread size, No. 139 nylon; frame lines, No. 120 braided nylon (net rigged on frame lines with No. 15 nylon seaming twine); basis, 5/9 (27 inches per foot); leads, 12 per pound hung at 1-foot intervals; floats, plastic. We usually order nets with square mesh sizes of 1-1/2, 2, and 2-1/2 inches.

So far the new nets have handled perfectly. We have hauled them hand over hand into boats, piled or stored them randomly, twisted them, and hauled them into tubs with no semblance of order. At no time have we had any trouble with tangling.

We believe that the key to such "free-wheeling" nets lies mainly in having an adequate basis (fullness of webbing) to keep the net from twisting and closely spaced leads on a braided lead line of sufficient diameter to make leads close fitting. The relatively large braided lead line alone may be, to a large degree, responsible for our success. Other features might be successfully altered to fit other fishing conditions.

-- CHARLES J. CHANCE and O. F. HASLBAUER, Tennessee Valley Authority, Norris, Tennessee.

AN EVALUATION OF BOUND DIETS

Bobby D. Combs and Roger E. Burrows
Bureau of Sport Fisheries and Wildlife
Entiat, Washington

THE EFFICIENCY OF DIET UTILIZATION is affected by two factors other than composition; namely, the loss of water-soluble components and the size of particles. Nutritionally adequate diets may prove deficient if the water-soluble components, both vitamins and proteins, are lost through leaching before they are consumed by the fish. A diet which breaks down readily because of the feeding action of the fish results in food particles so small as to be unavailable. In order to obtain maximum efficiency, the diet must be prepared and presented to the fish in such a manner as to prevent the loss of nutrients in the water. A diet which meets these qualifications is said to be well "bound."

The various components of the diet may be bound by the use of natural or artificial binders. Natural binders, as defined in this report, refer to meat products such as spleen, beef lung, hog liver, and salmon milt, which, when combined with salt, form a rubbery mass. Artificial binders are defined in this report as commercial nonmeat products. Several nonmeat products have been tested as binders with varying degrees of success. Gelatin has been used by many investigators. Wolf (1951) used a combination of gelatin and cooked potato starch to bind synthetic trout diets; he had found tapioca, Mucilose (psyllium seed), and vegetable gums unsatisfactory. Wood, Griffin, and Snieszko (1954) reported that CMC (carboxymethylcellulose) produced a better bind than fresh beef spleen and salt. Irish moss and Kelcoloid, which were also tested by these authors, were less efficient than CMC.

The diet-binding experiments at the Salmon-Cultural Laboratory at Entiat, Washington, during 1956 were divided into two phases. In the first phase, a series of physical tests was conducted to determine the optimum amounts of several commercial binders to be added to a test diet. The second phase consisted of feeding trials made to compare the growth rates of fish fed an unbound diet, a diet bound with salt, and seven diets bound with commercial binding agents.

Physical Tests

In order to determine the optimum amounts of various commercial binders for addition to the diet, a physical method of determining the degree of bind was desirable. After numerous experiments, a fast and unbiased photocolorimetric method was devised.

All of the commercial binding agents tested were furnished gratis by the manufacturers or distributors. Those tested included the following: three cellulose products (CMC, Ethulose C, and Methulose); two alginates (Kelcosol and Kraystay); three hydrophilic polymers (CB-35, K-707, and Carbopol 934); and Jaguar, a natural gum obtained from the guar seed. The names and addresses of the companies supplying these products will be furnished by the authors upon request.

The diet used to test the binders consisted of 10 percent beef liver, 20 percent hog liver, 10 percent beef lung, 10

percent arrow-toothed halibut, 40 percent salmon viscera, 5 percent seal meal, and 5 percent distiller's solubles. For each test, 100-gram lots were used. The dry binders were combined with the meal portion of the diet and added after the meats were mixed. The two liquid binders, CB-35 and K-707, were added after all diet components were mixed.

A standard procedure was followed for all physical tests: (1) The lots were allowed to stand at least 30 minutes after mixing; (2) with a small hand ricer, 40 grams of a diet were introduced into a measured amount of water contained in the bowl of a food mixer; (3) the water and feed were agitated by the planetary action of the mixer for 30 seconds at low speed; and (4) a water sample was then taken from the bowl, and color change and turbidity were read simultaneously on a photocolorimeter. Turbidity was due to a breakdown of food particles, whereas color change was due to the leaching of some food elements. A low reading would indicate that little breakdown and leaching had occurred, and that the diet was well bound.

Each of the binders was tested at the 1- and 2-percent level alone and in combination with 1 and 2 percent salt. Thus each binding agent was tested in six combinations. The combination that resulted in the highest degree of bind, as indicated by the lowest photocolorimeter reading, was selected for the feeding trials. In every instance it was necessary to include 2 percent of the binding agent to produce the maximum bind, but the amount of salt varied with the binding agent. It should be stressed that consistency was not the criterion employed in the selection of the binding combination used in the feeding trials but rather the bound quality of the diet as measured by the photocolorimetric readings. Because the experiment was limited to nine trials, two of the cellulose products -- Ethulose C and Methulose -- were eliminated from the test. These two products resulted in binds that were comparable to CMC but were more expensive in initial cost.

Feeding Trials

The physical tests provided a basis for the selection of the most efficient binding combinations. The feeding trials were an evaluation of the effectiveness of these combinations as measured by fish growth.

Chinook salmon (Oncorhynchus tshawytscha) fingerlings that averaged 117 per pound were stocked into 6-foot circular tanks at the rate of 2,270 grams (5 pounds) per tank. Each diet was fed to duplicate tanks of fish for 10 weeks. The fish were weighed at biweekly intervals, and the amount of food given was adjusted according to the weight of the fish and the water temperature. No changes in the number of fish per tank were made except those caused by mortality.

The basic diet fed was similar to the one used for the physical tests. It consisted of 12.5 percent each of hog liver and beef lung, 25 percent arrow-toothed halibut, 40 percent salmon viscera, and 5 percent each of seal meal and distiller's solubles.

The experiment contained nine variations of the test diet. One diet was fed unbound, one diet was bound with salt, and seven diets were bound with commercial binders in combination with salt. The binding agents and amounts of each may be seen in the table.

All diets were prepared daily, and the same mixing procedure as described for the physical tests was followed. The fish were fed with a hand ricer 3 times a day for the first 2 weeks and 2 times a day thereafter. The results of the feeding trials are summarized in the table.

Discussion

The results of the feeding trials show a striking contrast between the efficiency of bound diets and that of an unbound diet. Even the diet containing the most expensive binding agent, which increased the cost of the diet 5 cents a pound, was more economical with regard to cost per pound of fish produced than the unbound diet. The most economical diet was diet 2, bound with salt alone. Compared to the

SUMMARY OF FEEDING TRIALS USING VARIOUS BINDING AGENTS

Diet composition:

Hog liver	12.5%
Beef lung	12.5%
Arrow-toothed halibut	25.0%
Salmon viscera	40.0%
Seal meal	5.0%
Distiller's solubles	5.0%

Chinook salmon

Initial number per pound: 117 fish

Diet No.	Experimental variable		Gain in weight in 10 weeks (grams)	Conversion	Cost of binding agent used per 100 pounds of diet	Cost per pound of diet	Cost per pound of fish
1	Unbound		3,677	4.55	----------	$0.0700	$0.3185
2	Salt	1/ 2	7,805	2.50	$0.04	.0704	.1760
3	CB-35 Salt	2 1	7,652	2.60	.68	.0768	.1997
4	K-707 Salt	2 1	7,215	2.75	.43	.0743	.2043
5	CMC Salt	2 2	7,473	2.66	1.54	.0854	.2272
6	Jaguar Salt	2 1	5,212	3.38	1.12	.0812	.2744
7	Kelcosol Salt	2 2	7,683	2.62	2.64	.0964	.2525
8	Kraystay Salt	2 2	8,164	2.48	3.04	.1004	.2490
9	Carbopol Salt	2 1	7,585	2.58	5.02	.1202	.3101

Least difference at the 5% confidence level: 398 grams.

1/ Binding agents added at the rate of pounds per 100 pounds of diet.

unbound diet, diet 2 yielded more than twice the gain in weight at approximately half the cost per pound of fish produced. All of the commercial binding agents showed better binding qualities than salt in the photocolorimetric tests; however, the feeding trials demonstrated that the salt-bound diet was utilized as effectively as any other.

All of the bound diets except diet 6 produced comparable gains. The fish that were fed diet 6 were significantly smaller than those fed the other bound diets, although the consistency of the diet was good and the fish consumed it readily. Though there were some differences in weight gain among the other seven bound diets, these differences were due to biological variation rather than to the effect of the binders. Mortalities were low in all lots.

At the conclusion of the experiment, 10 fish from each diet were examined for evidence of nutritional deficiencies. These examinations included determinations of the hemoglobin content of the blood, the amount of fat deposition, and the color of liver, spleen, and kidney. None of the fish showed a tendency to anemia. Amounts of hemoglobin averaged 7.74 to 8.33 grams per 100 milliliters of blood; fat deposition appeared less than normal, the fat being confined to a strip along the intestine with the pyloric caeca free; and the livers, spleens, and kidneys showed a normal dark-red coloration.

The consistency of the bound diets varied with the binding agents employed. Kelcosol and Kraystay produced rubbery binds very similar in consistency to the salt bind in diet 2. The two liquid binders, CB-35 and K-707, produced feeds of a rather soft consistency, well bound yet very easy to handle and feed. On the other hand, CMC and Carbopol produced consistencies which were very stiff and difficult to feed. Smaller amounts of the binders in these diets would have produced better feeding consistencies, but the amounts used were necessary to prevent leaching. Diet 6, containing Jaguar, was rather gummy though fairly easy to feed. All of the diets floated when fed if they had been well mixed.

One advantage to the use of commercial binders is that less skill in mixing is required to obtain a bound diet. When salt alone is used in combination with meats, the salt must be added when the feed is at the proper temperature. Furthermore, mixing must be discontinued when the desired bind is produced, as additional mixing will destroy the bind. The commercial binding agents tested in this experiment bound the diet whether it was very cold or had warmed considerably. Also, the bind was quite resistant to breakdown by mixing.

The diet bound with salt alone was the most efficient in terms of both cost and growth rates. On the basis of these experimental results, it would be difficult to justify the use of commercial binders except in diets which cannot be bound with salt. Here, again, the formulation of such diets would be difficult to justify except on an experimental basis. The addition of hog or beef spleen, beef lung, or hog liver, or combinations of these components at approximately the 20-percent level, will bind most diets adequately when combined with salt. None of these products is excessive in cost or difficult to obtain. It has been demonstrated at this laboratory that any of these natural binding agents can be incorporated in combination diets with excellent results. In the formulation of diets for the production hatchery, salt in combination with meat binding agents offers the most economical solution to the feeding problem.

Summary

1. A photocolorimetric method of measuring the maximum binding ability of various binding agents has been developed and the method applied in the development of bound diets for feeding trials.

2. Feeding trials, conducted for 10 weeks, demonstrated that a 100-percent increase in food utilization could be attained by using adequately bound diets.

3. Salt, at the 2-percent level, proved as efficient as any of the commercial binding agents in the test diet.

4. The cost of commercial binding agents, in comparison with that of salt, makes their use difficult to justify in normal production operations.

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To further the work of the International North Pacific Fisheries Commission in differentiating the various North Pacific salmon stocks from Asiatic salmon stocks by analysis of their racial characteristics, the Bureau of Commercial Fisheries has recently developed a device--using strobe illumination and two cameras--which will provide body measurements that are accurate within $\pm$ 0.05 centimeter when ideal subjects are measured.

URETHANE AND CANCER

Recently the status of occupational carcinogens as related to the fishery professions has been reviewed briefly in this office with Dr. W. C. Hueper, Chief, Environmental Cancer Section, National Cancer Institute, at Bethesda (14), Maryland. Dr. Hueper has reminded us of his continuing interest in receiving information about the occurrence of cancer in fish. He would appreciate getting verified reports of tumors, growths, or other cancerous conditions in fish. Also, he would be glad to receive suitably preserved specimens.

This subject was first brought to the attention of fishery workers by E. M. Wood's "Urethane As a Carcinogen" (THE PROGRESSIVE FISH-CULTURIST 18 (3): 135-136). The evidence concerning the role of urethane as a cancer-producing agent in experimental animals continues to mount. The potential hazard of this drug to those who employ it cannot be overemphasized.

In the interest of fostering a conservative approach to the use of this chemical, a part of the editorial note that accompanied Dr. Wood's article is reprinted here:

> ... there is a possibility that humans might get cancer from the use of urethane. It is suggested, therefore, that anyone who works with urethane should do so with the utmost caution. Rubber gloves should be worn to protect the hands, respirators should be used when the powder is being weighed for the preparation of solutions, and some precaution should be taken to protect the body from water that is splashed by fishes.

Better still: use a suitable substitute.

-- The EDITOR.

THE USE OF MALACHITE GREEN TO CONTROL *TRICHODINA*

Wallace F. Hublou
Oregon Fish Commission
Clackamas, Oregon

IN THE FALL OF 1955, at the Bonneville Hatchery of the Oregon Fish Commission, a pond of fingerling steelhead trout (Salmo gairdneri gairdneri) was discovered to be infected with the peritrichous ciliate, Trichodina. This genus of protozoan is parasitic or commensal on a wide variety of fauna (Uzmann and Stickney 1954). When found in association with fish, it is considered ectoparasitic on the gills and body surfaces. In this pond, the fish were very heavily parasitized with Trichodina and considerable losses were occurring as a result. Ordinarily, the Trichodina would not have posed much of a problem, as we have found that it is easily controlled with a 1:4,000 solution of formalin under normal hatchery conditions. However, the Bonneville pond was of irregular size and had a capacity of approximately 8,800 cubic feet of water. The pond was constructed of dirt and could not be cleaned readily, nor could the water level be lowered safely without first removing the fish. The fish were quite weak as a result of the heavy infestation, and it was not desirable to handle them in this condition. The use of formalin was impractical because of the amount required to treat fish in such a large volume of water. Also, it would have been inadvisable to use formalin because the pond could not be flushed at the end of the treatment.

This report describes three experiments designed to determine the effect of malachite green on Trichodina and parasitized fish. It also contains a discussion of the successful application of the results to a group of infected hatchery fish.

Procedure

Trial 1 was conducted in hatchery troughs, and three species of salmonoids--silver salmon (Oncorhynchus kisutch), fall chinook salmon (O. tshawytscha), and steelhead trout--were used as test animals. Two different concentrations of a malachite green solution were used to check the toxicity of this material on the fish and to determine whether the fungicide in the concentrations used would control the parasite present only on the steelhead. Trials 2 and 3 were conducted subsequently in the laboratory, using excised fins infested with Trichodina, to observe the toxic effect of various concentrations of malachite green on Trichodina. Formalin was also tested in trials 1 and 3 to serve as a comparison for the results obtained from the malachite green treatments.

Trial 1 was conducted in four hatchery troughs. One trough was used as the fresh-water control. Two troughs contained solutions of malachite green: one at a concentration of 1:150,000 and the other at a concentration of 1:375,000. The fourth trough contained a solution of 1:4,000 formalin. Each of the troughs was stocked with 100 steelhead averaging 0.5 gram (900 per pound), 25 silver salmon averaging 15.1 grams (30 per pound), and 25 fall chinook salmon averaging 11.4 grams (40 per pound).

All the fish of each species were placed in separate baskets in the troughs for the treatment and were held there 24 hours after being treated. They were examined

before the treatment and 24 hours later. Water intakes were left on in the troughs treated with malachite green, and the chemical was introduced by the drip method with a constant-siphon jug (Burrows 1949). Formalin was mixed in the water at the start of the treatment, and the intake was shut off. The intake of the control trough was also shut off during treatment. The water temperature was 45° F. The water level in the troughs was maintained at the normal 6-inch depth, and the treatment in each trough was 1 hour in duration.

Trials 2 and 3 were conducted in the laboratory with parasitized fins which had been excised from infected fish. The fins were placed in petri dishes in which a measured amount of the treatment solutions had been prepared. In trial 2, malachite green was used in 7 different concentrations that ranged from 1:200,000 to 1:800,000. In trial 3, malachite green was used at 3 concentrations: 1:200,000, 1:400,000, and 1:800,000. In trial 3, a 1:4,000 solution of formalin was also tested. A fresh-water control was used in both trials. The temperature of the hatchery water supply was 43° F., and the temperature of the solutions in the petri dishes did not exceed 48° F. at any time during the trials. The parasites were observed by placing the petri dishes on the stage of a dissecting microscope at 10-minute intervals during the experiments. A magnification of 45x was used to observe the protozoans. The fins were not removed from the solutions while under observation. After the fins had been in the solutions for 90 minutes in trial 2 and 30 minutes in trial 3, the solutions were drained off and fresh water was added to the petri dishes. This was done in order to facilitate observation of any recovery that might occur in the protozoans which had been exposed to the malachite solution. Trichodina activity was categorized in the following four levels: active, sluggish, no movement but with cilia still beating, and dead.

Results

The results of trial 1 show that very small fingerling steelhead, which were in a weakened condition due to a heavy infestation of Trichodina, suffered an 85-percent mortality when subjected to a 1-hour treatment with malachite green at a concentration of 1:150,000 (table 1). In a more dilute concentration of 1:375,000, the loss was 10 percent. A treatment with formalin at a concentration of 1:4,000 resulted in a 10-percent mortality. The control lot in fresh water suffered a 2-percent mortality. Silver and fall chinook fingerlings (considerably larger than the steelhead), which were not infected with Trichodina, suffered no mortality at any of the treatment levels. All the mortalities were noted 24 hours after treatment. The 3 treated groups of steelhead had only

TABLE 1.--Fish mortalities and Trichodina activity after treatments with malachite green at various levels and formalin in trial 1 [1]

Treatment (1 hour)	Percentage fish mortality 24 hours after treatment			Trichodina on steelhead 24 hours after treatment	Trichodina activity 24 hours after treatment
	Steelhead trout	Silver salmon	Fall chinook salmon		
Fresh water (control)-------	2	0	0	Heavily infected	Very active.
Formalin at 1:4,000---------	10	0	0	Very few	No movement.
Malachite green at 1:150,000-------	85	0	0	---do.----------	Do.
1:375,000-------	10	0	0	---do.----------	Do.

[1] Only the steelhead were infected with Trichodina.

TABLE 2.--Activity of Trichodina on excised steelhead fins after treatment with malachite green at various levels in trial 2

Treatment	Parasite activity during treatment[1] (minutes)									Percentage recovery of Trichodina after 90-minute treatment and 60 minutes in fresh water
	10	20	30	40	50	60	70	80	90	
Fresh water (control)-------	A	A	A	A	A	A	A	A	A	100
Malachite green at										
1:200,000-------	A	A	S	N	N	N	N	D	D	0
1:300,000-------	A	A	S	S	N	N	N	D	D	0
1:400,000-------	A	A	S	S	N	N	N	D	D	0
1:500,000-------	A	A	S	S	N	N	N	D	D	0
1:600,000-------	A	A	S	S	N	N	N	D	D	0
1:700,000-------	A	A	S	S	N	N	N	D	D	0
1:800,000-------	A	A	A	S	S	N	N	N	D	0

[1] Protozoan activity: A = Active. S = Sluggish. N = No movement, cilia still beating. D = Dead.

a few motionless Trichodina remaining, whereas the control group was still heavily parasitized with active protozoans.

Trial 2 seems to indicate that malachite green, at concentrations of 1:200,000 to 1:800,000, will result in a complete kill of Trichodina when treated for 90 minutes (table 2). When first put into the solutions, the Trichodina were very active, some attached on the fins and some free swimming. Movement was reduced as the solutions of malachite green apparently began to affect the protozoans. When the cilia stopped beating, the Trichodina were assumed to be dead. After death, the Trichodina which had been attached to the fins came loose and settled to the bottom of petri dishes.

The results of trial 3 indicate that a treatment with malachite green for 30 minutes at concentrations of 1:200,000 and 1:400,000 was sufficient to kill all of the Trichodina eventually, although not during the treatment itself; whereas at a concentration of 1:800,000, the malachite green killed approximately 95 percent of the protozoans (table 3). Formalin at a concentration of 1:4,000 was very successful in killing Trichodina, as it killed them in 10 minutes or less. A repeated trial produced the same results.

On the basis of the results of the various experiments, it was decided to treat the Bonneville pond containing approximately 300,000 infected steelhead with

TABLE 3.--Activity of Trichodina on excised steelhead fins after treatment with malachite green at various levels and formalin in trial 3

Treatment	Parasite activity during treatment[1] (minutes)			Percentage recovery of Trichodina after 30-minute treatment and 60 minutes in fresh water
	10	20	30	
Fresh water (control)-------	A	A	A	100
Formalin at				
1:4,000---------	D	D	D	0
Malachite green at				
1:200,000-------	A	A	S	0
1:400,000-------	A	A	S	0
1:800,000-------	A	A	S	5

[1] Protozoan activity: A = Active. S = Sluggish. D = Dead.

malachite green for 1 hour. As the water level could not be lowered with the fish in the pond, the malachite green solution would have to be allowed to dissipate naturally after the treatment. To avoid overexposing the fish to the malachite green solution, the water inflow of the pond was adjusted to produce a concentration of 1:800,000 by the end of the 1-hour treatment. This was assuming thorough mixing and no loss of malachite during the treatment. The actual concentration of the treatment was somewhat less than this because, owing to poor circulation within the pond, some of the solution was lost before the end of the treatment. The green color of the solution was not observable 4 hours after the treatment. The treatment was a success, resulting in control of the protozoans with a very low immediate fish mortality.

Conclusions

Malachite green can be used to control Trichodina. With the conditions under which the tests were made, malachite green in concentrations of 1:200,000 to 1:800,000 killed Trichodina when subjected to a 90-minute treatment. Applied in a 30-minute treatment followed by a 60-minute period in fresh water, concentrations of 1:200,000 and 1:400,000 resulted in a complete kill and a concentration of 1:800,000 resulted in a kill of approximately 95 percent.

A 1-hour treatment with malachite green at a concentration of 1:150,000 resulted in an 85-percent mortality of small (900 per pound) steelhead trout which were heavily infected with Trichodina.

A field test in which malachite green was at a maximum concentration of somewhat less than 1:800,000 by the end of the 1-hour treatment, and was then allowed to dissipate naturally, resulted in control of Trichodina and very low fish mortality from the treatment. The treatment was administered to 300,000 fish averaging 900 per pound in a dirt pond which had a capacity of 8,800 cubic feet of water.

Acknowledgments

The author wishes to acknowledge that use of malachite green to control Trichodina was suggested by A. W. Anderson, superintendent of the Bonneville Hatchery. He also wishes to express sincere appreciation to T. B. McKee for advice and assistance in preparing this report.

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For use in studies on the estuarine phase of the life cycles of Penaeus setiferus and P. aztecus, a pushnet has been designed to yield quantitative samples while being operated by one man in stiff, rooted vegetation in depths of 3 to 18 inches.

During 1957, as part of the Dingell-Johnson program for sport fish restoration, 42 States and 3 Territories obligated $2,103,800 for 150 investigational or fact-finding studies. Principal emphasis was placed on the inventory of fish populations and the determination of population characteristics, rate of harvest, and angling pressure.

NATURAL RESISTANCE AND SUSCEPTIBILITY TO INFECTIONS

S. F. Snieszko
Bureau of Sport Fisheries and Wildlife
Eastern Fish Disease Laboratory, Kearneysville, West Virginia

THE PURPOSE OF THIS ARTICLE is to outline briefly the stages in the development of research on natural resistance to infectious diseases, with reference to the study of infectious diseases in fresh-water fishes.

We are beginning to realize that among animals there are populations, strains, or individuals which are not susceptible all of the time, or even temporarily, to some of the infectious diseases. There must be reasons for this, and some of the reasons have been or are being discovered (Dubos 1954). The recently observed facts about "natural resistance" are opening new avenues to the better control of disease. We are beginning to understand this problem, and research on diseases of fish may be a particularly rewarding field of investigation. Cold-blooded animals may serve as excellent material for research that will throw a new light on seasonal variation in the degree of "natural resistance," not only in fish but perhaps even in higher vertebrates (Cushing and Campbell 1957).

During the past century it has been generally assumed that all that is necessary for an outbreak of an infectious disease is an intimate contact between a pathogenic organism and a representative of an animal or plant species normally susceptible to such a disease. Dubos (1955) offered an excellent criticism of this idea. He said that the widely accepted view of the relation between patient and microbe is too oversimplified to fit the facts of a disease more than rarely. He emphasized what most of us know--that there are many situations in which the microbe is always present but causes disease only under certain special circumstances. He goes on to state that the fact that any population survives and flourishes even in the presence of potentially pathogenic microbes implies that members of the population have a high degree of natural resistance.

Burnet's book on epidemiology (1953) is a well-written, simply presented work; anyone who has to deal with infectious diseases of animals will profit by reading it. Burnet makes it clear that there are many factors contributing to outbreaks of infectious diseases.

By the turn of the century it had become obvious that treatment of infectious diseases with the drugs available at that time was generally ineffective. Fortunately, immunization with attenuated or dead pathogens, or by injection of specific immune sera, permitted effective control of several infectious diseases. Recognition and introduction of sanitation further reduced the danger of dissemination of pathogens and the likelihood of infection.

Chemotherapy, which has made its greatest progress just during the past 20 years, is the massive use of drugs that are more toxic to the pathogens than to the host in which they cause a disease. Use of quinine, a natural chemotherapeutic drug for effective treatment of malaria, was an early application of this method. More recent drugs used in chemotherapy are the sulfonamides, which are synthesized by chemists; others, the antibiotics, were and still are chiefly isolated from

micro-organisms. Sanitation, immunization, and chemotherapy have provided the means for reducing mortalities caused by infectious diseases. Less than a century ago these same infectious diseases were responsible for more than half of all deaths, including those from old age. Despite the progress made, infectious diseases still are responsible for the death or crippling of people and animals and for reduction of agricultural crops. Drugs are most commonly used to treat outbreaks of disease; they are less commonly used prophylactically, to prevent outbreaks. Unfortunately, even with today's advanced methods, not all outbreaks of disease are curable with chemotherapy. For one thing, the disease may not be recognized in time for treatment and the drugs may be given too late; for another, the pathogens may prove drug-resistant.

Not all scientists in the past have accepted implicitly the belief that outbreaks of sickness resulted solely from exposure to micro-organisms. Probably the most spectacular demonstration was made by Pettenkoffer, a German hygienist, who drank a cupful of freshly isolated cholera germs to prove that under certain conditions this deadly pathogen would not cause the disease. As he expected, Pettenkoffer did not contract cholera.

Before going further, the term "natural resistance," as used in my title and in this article, requires definition. In a symposium on natural resistance to infections (McDermott 1956), Schneider differentiated the resistance between host species (interspecific) and the resistance within host species (intraspecific). Recently a considerable amount of research and speculation has dealt with the natural intraspecific susceptibility or resistance to diseases. McDermott's symposium, as well as one edited by Snieszko (1954), and papers by Flemming (Schäperclaus 1956: 8), Bearn (1956), Plančić (1956), Svet-Moldawsky (1954), and Kaplan and Crouse (1956) have provided additional incentives for writing this article. It is well known that warm-water fish like carp, which are grown commercially in ponds in countries within the temperate zone, go through two growth phases each year. There is a period of rapid growth when the temperature of the water is about 60° to 90° F., and a period of low activity and metabolism at lower temperatures. Studies (Snieszko 1954) have shown that at lower temperatures antibody production of carp in response to injections of antigen is greatly reduced. This depressing effect of low temperatures seems less pronounced in cold-water fish like trout, which metabolize actively at temperatures of 40° to 50° F. (Smith 1940).

Flemming (Schäperclaus 1956: 8) found that the reduction of total blood protein amounted to as much as 45 percent in carp that overwintered. Plančić (1956) reported that hemoglobin in carp blood dropped 40 to 50 percent during the winter and early spring. The greatest decrease occurred in April, when the carp became active but little natural food was available. Bacterial diseases, like infectious dropsy, rise in spring and cause heaviest losses when the temperature of water rises. It seems likely that fish blood is then deficient in antimicrobial components. It is interesting that temperature also affected the production of virus-neutralizing antibodies in reptiles (Svet-Moldawsky 1954).

Offhaus, Brunner, and Riedmüller (1955) demonstrated, in a series of very interesting experiments, correlation between the health of carp and the quantitative relationship between different protein fractions of the blood serum.

Miscellaneous factors influencing natural resistance and the role of nutrition in infections were discussed in two recent symposia (McDermott 1956, Wright 1955). Particularly noteworthy among the topics discussed in these two symposia was the discovery of a new blood component called properdin, the presence or absence of which in blood has a considerable influence on nonspecific resistance to infection.

Bearn reported (1956) that agammaglobulinemia, a hereditary disease in which the antibody-containing fraction of blood serum is absent or nearly absent, is characterized by much greater susceptibility to infectious diseases. Williams (1956) reported that a number of other

biochemical components of hosts and parasites are linked with genes, and thus that the potential resistance or susceptibility to infectious diseases can be inherited.

Wolf (1954) and Snieszko (1957) demonstrated that hatchery strains of eastern brook trout (Salvelinus fontinalis) had differences in susceptibility to furunculosis and ulcer disease. This is interpreted as an expression of intraspecific genetic difference. It is most likely that these genetic differences could be traced to biochemical differences in the makeup of blood--differences that can be detected with modern methods. If these differences can be detected, remedial measures may be tried next. Research in biochemical genetics and nutrition probably will furnish us with additional approaches to the control of fish diseases. The fact that one strain of fish may be more susceptible than another to a disease does not necessarily mean that the more susceptible strain should be eliminated, especially if it has other highly desirable characteristics. Williams (1956) summarized this situation as follows:

> It is by no means impossible to deal with diseases which have a hereditary basis. We do so every time we use insulin or thyroid tablets, correct nearsightedness or farsightedness with spectacles, or have dental work done; and it is ridiculous to suppose that knowing about the hereditary basis of diseases will not help us cope with them.

During recent years I have observed that this laboratory has received large numbers of samples of sick warm-water fish during the spring. Usually the first samples of the year are received in February or March from the States on or near the Gulf of Mexico. In April, most of the samples are received from Arkansas, Tennessee, Georgia, and the Carolinas; in May and June, the samples are received from the States farther north. This indicates that the highest susceptibility of warm-water fish to bacterial infection in the United States follows the same course reported by European investigators (Schäperclaus 1954, Snieszko 1954). At the Leetown laboratory, Aeromonas liquefaciens, believed to be the agent of carp dropsy in Europe, has been isolated from many of the warm-water fish submitted to us for examination. Similar pseudomonads have been isolated from fish with apparent low-grade infections--infections which may erupt explosively and cause heavy mortalities.

Obviously, fish and other cold-blooded animals could serve as exceptionally good experimental animals for research on the nature of natural resistance to disease. This research would be largely biochemical in nature, but the cooperation of bacteriologists and serologists would be essential. There is little doubt that the results of such research would contribute to the better control of infectious disease.

One fallacy which is sometimes encountered in fish culture is the assumption that when a certain fact is discovered in the laboratory, it may be generalized and indiscriminately applied to different species or strains of fish existing in different ecological conditions and of different age. Just as there is no average human, there is no such thing as an average fish. Before the results of any scientific discovery are generalized, additional exacting and repeated experiments are necessary to determine the degree to which the generalization is permissible. Williams (1956), from whom I have already quoted, has this to say on this point:

> Of course we can draw a single recognizable picture of man. He will have a skeleton, muscles, organs, nervous system, hungers, emotions, aspirations, etc., but as long as we hope to solve or understand human problems on the basis of such a generalized being, our operations must be at a very elementary level and many problems will completely elude us.

Williams might just as well have said this about fish.

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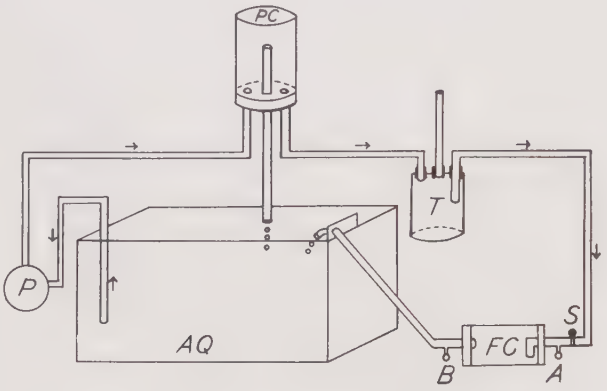
A METHOD FOR MEASURING THE OXYGEN CONSUMPTION OF FISH

Paul O. Fromm
Department of Physiology and Pharmacology, Michigan State University
East Lansing, Michigan

MANY MEASUREMENTS OF THE OXYGEN CONSUMPTION OF FISH have been made in the past with equipment ranging in complexity from the Warburg apparatus to simple jars in which fish were kept for varying periods of time. The results that Shlaifer (1938) reported for Carassius auratus and that Geyer and Mann (1939) reported for Perca fluviatilis indicate the desirability of measuring the oxygen consumption of individual fish rather than that of groups of fish. If reliable results are to be obtained, it is necessary to have a constant-flow apparatus to carry away various metabolic waste products which may affect the general metabolic rate. To study the effect of toxic materials on the oxygen consumption of fish, the apparatus shown diagrammatically here was constructed. This apparatus is a rather drastic modification of that used by Keys (1930) but embodies the same principle.

Description of Apparatus

Water is pumped from the aquarium, AQ (see diagram), to a pressure chamber, PC, by a motor-driven centrifugal pump, P, made of monel metal. If desired, a rheostat can be used to control the speed of the pump so that a small excess of water returns to the aquarium via the standpipe, thus maintaining a constant head of pressure. The rate of flow from the pump



Apparatus for measuring the oxygen consumption of fish. A, end of T-tube with rubber serum-bottle stopper; AQ, aquarium; B, same as A; P, monel metal centrifugal pump; PC, pressure chamber; S, adjustable pinchcock; T, bubble trap. Arrows indicate the direction of water flow. (Not drawn to scale.)

can also be regulated by placing an adjustable pinchcock between the pump and the pressure chamber. Water from the pressure chamber flows into the trap, T, which prevents the entry of any bubbles of air into the fish chamber, FC. It has been found desirable to have on the top of the fish chamber a small opening (not shown in the diagram) through which bubbles of air can be removed, as it is virtually impossible to place the fish in the chamber and replace the stopper without trapping some air in the chamber. The rate of flow through the fish chamber is controlled by an adjustable pinchcock, S. In practice, there are four fish chambers arranged in parallel rather than a single

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chamber as shown in the diagram. Water enters the fish chamber at a right angle to the direction of flow, minimizing the buildup of a current against which the fish must swim. The fish is always oriented "upstream" in the chamber, and the water flowing from the chamber returns to the aquarium. Connections between the parts of the apparatus are glass and tygon tubing, and the only contact that the water in the system has with metal is in the monel metal pump.

Measurement of Dissolved Oxygen

The sodium azide modification of the Winkler method is used for the determination of dissolved oxygen. To permit a large number of measurements of oxygen consumption of fish exposed to a known type of water, a semi-micro method for measuring dissolved oxygen was developed. The $MnCl_2$ solution, alkaline KI solution, and phosphoric acid are convenient for use if kept in rubber-stoppered serum bottles of 40- to 60-milliliter capacity. Approximately 8.5 milliliters of water is drawn into a 10-milliliter syringe, and then 0.5 milliliter is expressed, making sure that no air bubbles are present with the 8 milliliters of water left in the syringe. Next, 0.2 milliliter of $MnCl_2$ solution, followed by 0.4 milliliter of alkaline KI solution, is drawn into the syringe. The needle is then inserted in a cork, and the sample is mixed by rotating the syringe. After about 5 minutes, 0.4 milliliter of concentrated o-phosphoric acid is drawn into the syringe, dissolving the precipitate and liberating the iodine. The contents of the syringe (9.0 milliliters) are expressed into a test tube, and the iodine is titrated with 0.005 N. thiosulfate by means of a syringe burette. Thus for each determination of the oxygen consumption, approximately 19 milliliters of water is removed from the system and the only contact of the water sample with air during the analytical procedure is at the end of the syringe needle.

Fox and Wingfield (1938) recommended use of o-phosphoric acid instead of sulfuric acid when doing microdeterminations of dissolved oxygen because the sulfuric acid causes liberation of some iodine from the alkaline iodide solution. The use of sulfuric acid in the ordinary Winkler method does not cause liberation of iodine from the alkaline iodide solution, presumably because of the greater volume of water sample and consequent greater dilution of the reagents.

Measurement of Oxygen Consumption of Fish

Experimental fish are anesthetized with a 0.03-percent solution of tricaine methanesulfonate (M.S. 222). Immersion in the M.S. 222 for 30 to 45 seconds is usually adequate to keep fish immobilized while measurements of length and weight are made. A fish is then inserted in the fish chamber with its head pointed "upstream." Water is allowed to run through the chamber at its maximum rate while the fish is recovering from the anesthesia, usually 10 minutes or less. A comparison of anesthetized and nonanesthetized fish has shown that the M.S. 222 apparently has no lasting effect on the general metabolic rate. It is necessary to let the fish acclimate to its new surroundings for at least 2 hours (preferably longer), as very high values for oxygen consumption are obtained for the first few hours after fish are placed in the tubes. After the acclimation period is complete, the rate of flow through the fish chamber is adjusted and measured with a graduated cylinder and a stopwatch. Samples of water are taken at A and B (see diagram) and measured for dissolved oxygen. The head of pressure is sufficient to fill the syringes slowly without applying any negative pressure on the plunger. If the syringe is filled at B in the manner described, the sample will consist of water that flowed past the gills at the measured rate; whereas taking a sample at B by strong negative pressure on the syringe plunger could alter the rate of flow through the tube and result in low oxygen-consumption values. Using the difference in dissolved oxygen (D.O.) between A and B and the rate of flow through the chamber, the oxygen consumption of the fish is calculated as follows:

$$\text{Oxygen consumption (ml./gm./hr.)} = \frac{\text{D.O. at A (ml./l.)} - \text{D.O. at B (ml./l.)} \times \frac{\text{flow (ml./min.)} \times 60}{1{,}000}}{\text{weight of fish (gm.)}}$$

Example of Application of Apparatus Described

By the method described, the resting metabolism of largemouth bass (Micropterus salmoides) was determined at 20° to 21° C. After the "control" oxygen consumption had been established, potassium chromate was added to the aquarium water to give a concentration of 94 p. p. m. Cr^{+6}. The dissolved oxygen content of the water never dropped below 5 p. p. m. during the experiment. The results are summarized in the table. The data for 49 and 68 hours' exposure to chromium are significantly lower than control data (to the 1-percent level) as determined by the Fisher "t" test. More information concerning the toxic action of hexavalent chromium on fish will be published at a later date; no attempt to evaluate the data presented in this paper will be made at this time.

EFFECT OF EXPOSURE TO 94 p.p.m. Cr^{+6} ON THE OXYGEN CONSUMPTION OF FOUR LARGEMOUTH BASS WITH AN AVERAGE LIVE WEIGHT OF 13.1 GRAMS

Treatment with 94 p.p.m. Cr^{+6} (hours)	Number of fish	Number of data	Oxygen consumption (ml./gm./gr.) Mean	Oxygen consumption (ml./gm./gr.) Standard deviation	Remarks
None	4	12	0.095	0.008	All fish o.k.
2.5	4	12	.105	.015	All fish o.k.
23.5	4	17	.102	.014	2 fish on back.
49.0	4	12	.074	.007	2 fish on back.
68.0	1	7	.069	.011	3 fish dead.[1]

[1] 3 fish died between 49-hour and 68-hour runs.

Conclusions

A method for measuring oxygen consumption of fish is described, and an example is included to show how the method can be used in studying the effect of toxic materials on the general metabolism of fish. All the materials needed to construct the apparatus can be obtained from scientific-instrument companies at a total cost of less than $200.

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A METHOD FOR MARKING FISH WITH AN ELECTRICAL BURNING DEVICE

George G. Fleener
Missouri Conservation Commission
Columbia, Missouri

WHEN MISSOURI BEGAN A STUDY to evaluate the effects of maintenance stocking of smallmouth bass, it was apparent that some method must be devised for marking permanently the planted fish. The accuracy of measuring the effects of stocking depends on the use of well-marked fish. The method used for marking must be speedy, must be as safe as possible for the fish, and must leave a mark which will permit positive identification of the stocked fish at a future date. Methods that were considered included the use of tattooing and the removal of fins by clipping and cauterization as described by Herman (1946). Complete removal of fins by searing, the method described here, more nearly meets the above requirements than any of the other methods considered.

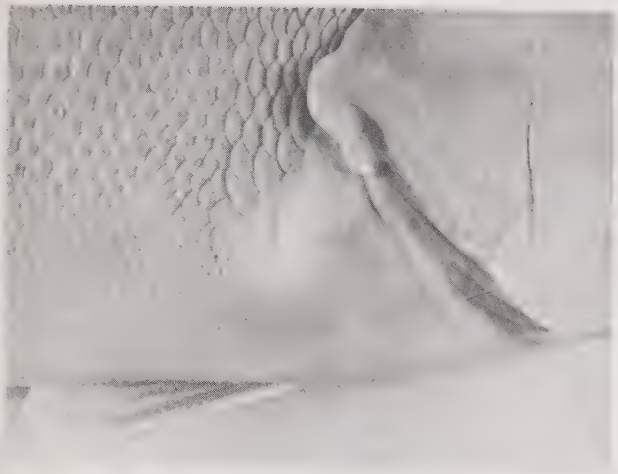
Procedure

In this technique a fin is removed completely by the use of an electrical searing tool. The searing tool consists of a high-resistance wire mounted in a handle. Its power is derived from a 6- to 12-volt source. The resistance wire is 23-gauge Chromel A. The wire is held by two prongs removed from a cord-connector cap. The screws in the prongs hold the resistance wire firmly in place and allow for rapid change of a burned-out wire. These two prongs are inserted into the cord-connector body. The cord-connector caps and cord-connector bodies are rated for 10 amperes at 115 volts. The handle is a 5-inch length of white pine shaped to fit the hand. A 3/8-inch hole through the handle accommodates 18-gauge drop-cord wire from the power source. One end of the handle is countersunk 1 inch deep to hold the cord-connector body. The power source varies under field conditions: it may be an auto battery or 115-volt alternating current. When the latter source is available, the current is changed with a transformer delivering 6 volts at 5 to 6 amperes.

During the marking operation, smallmouth bass fingerlings averaging 2 to 6 inches in length are anesthetized. This speeds up the work and reduces mortality by decreasing the handling time. The operator sears off the fin by touching it near its base with the searing tool. Care is needed to insure complete removal of the fin. It has been found that frequent rest periods for the operators cut fatigue and reduce injury to the fish. A tired operator often fails to remove all of the fin and often burns the fish.

Results

For our use speed is a requisite, as the entire stocking operation is accomplished within a few days, usually in hot weather. In 1952, a total of 4,675 fingerling smallmouth bass were marked at the rate of 245 per hour per operator. During 1953, the same operators marked 23,550 fish at an average rate of 294 per hour--an expenditure of 80 man-hours of labor. In 1954, the operators marked 37,880 fish



(Above) The stump of the marked right pectoral fin of a smallmouth bass one year after marking.

(Right) The electrical burning tool.

(Photographs by courtesy of Charles A. Purkett, Jr.)

at the rate of 317 per hour. This increase in the average number marked per unit of time reflects an increase in the skill and proficiency of the operators.

Few bass are killed by marking in this way. All of the marked fish are held approximately 18 hours in concrete live tanks before being hauled to the stocking sites. During 1953, smallmouth bass fingerlings marked in this manner had a survival of 96.8 percent at the end of the holding interval. These fish were handled when air and water temperatures were abnormally high. In view of the rigorous condition under which the work was done and the possibility of mortality from other causes not directly referable to the marking operation, the total loss is considered very small.

The greatest value of this technique is the elimination of fin regeneration. On July 15, 1953, a total of 112 fingerling smallmouth bass were marked and placed in a pond near Columbia. They were held until July 23, 1954, when the experiment was terminated because of the low water level in the pond. Of these, 24 fish were recovered and all 24 were still perfectly marked. There was no regeneration of the seared fins.

This experiment was not intended to obtain a check on survival, as other factors are believed to have influenced the survival of fish held in this pond. The experiment did provide evidence of the permanence of the mark on all of the fish recovered. Other experiments that have been conducted since 1952 have substantiated these results. Marked fish which have been recovered with the creel from the stream in which a stocking experiment is being conducted have shown no evidence of regeneration of the marked fin.

As this marking technique has proved successful, the effects of maintenance stocking of fingerling smallmouth bass can be evaluated in terms of return to the creel.

Acknowledgments

The author wishes to express his sincere thanks to H. L. Rowe, who in large

part designed the burning tool and provided invaluable assistance in many of the details pertaining to our marking operation. J. L. Funk also made many helpful suggestions during the course of our work.

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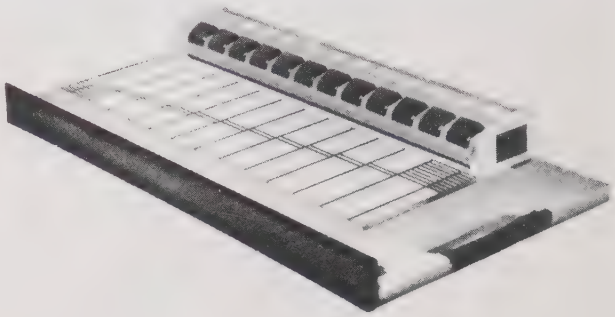
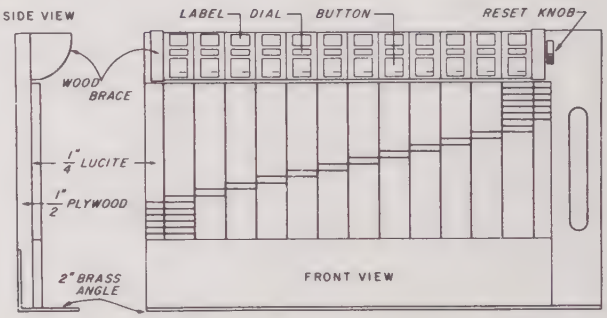
A RECORDING - MEASURING BOARD

The necessity of measuring large numbers of sea scallops, both ashore and at sea, to obtain basic data on the size composition of the commercial catch has led to the development of a new type of measuring board. It is possible to measure a sample of 500 shells in an hour with this board, whereas only 75 to 100 per hour can be measured with calipers.

The photograph and drawing show all the features of the board except a piece of clear plastic film that is fastened over the bank of counters with waterproof plastic electrician's tape to protect them from salt-water splash and spray. The face of the board, made of acrylic plastic because of its good dimensional stability, is scribed with 12 vertical columns. Within each column are 2 horizontal lines that define the upper and lower limits of a 5-millimeter size-group. (The scribing was done with a circular saw; the grooves were filled with black paint.) At the top of each column is a button-operated counter; the 12 counters are on a common reset shaft.

In use, the hinge of a scallop is placed firmly against the brass index stop and slid along it until the opposite margin of the shell falls within a box. The button at the head of that column is pressed to register one specimen in that size-group. When the entire sample has been measured, the frequency distribution is copied from the counter dials and each of them is reset to zero by turning the knob. Any shells that fall outside the range of sizes represented by the counters are measured by using the scales at either end of the board.





The board offers a number of advantages in addition to decreasing the time required to measure a large sample of shells. Gloves may be worn during cold weather. Fatigue of the operator, one source of error, is reduced. Errors incident to reading a scale and remembering and writing down a number are reduced. The labor and possibilities of error while transcribing, compiling, and tabulating data are reduced.

The only sacrifice, apart from the cost of the board (about $75), is the loss of the individual measurements.

--JULIAN A. POSGAY, Bureau of Commercial Fisheries, Woods Hole, Massachusetts.

LIGNASAN AS BACTERICIDE AND ALGAECIDE

Roger E. Burrows and B. D. Combs
Bureau of Sport Fisheries and Wildlife
Salmon-Cultural Laboratory, Entiat, Washington

THE EXPERIMENTATION RESULTING IN THE DEVELOPMENT OF LIGNASAN (ethyl mercury phosphate, 6.25 percent) for the control of bacterial gill disease, reported by Rucker, Earp, and Burrows (1956), has been continued at the Salmon-Cultural Laboratory. Coincidently with these investigations, it was observed that Lignasan was also an effective algaecide. As this laboratory has been searching for an effective algaecide for the control of green algae in rearing ponds, this fortuitous development has been further explored.

The routine procedure for the evaluation of a chemical as a potential algaecide employs simultaneous tests to determine the maximum concentration that the fish will tolerate for a 90-minute exposure period and the minimum concentration with algaecidal properties. Obviously, if the two concentrations do not overlap, the chemical must be discarded.

The tolerance level with Lignasan had been determined previously. The algaecidal tests, however, indicated that a concentration of 1:1,000,000 was adequate for the control of green algae. Tests were then conducted to determine the frequency of treatment necessary to control algae under simulated production conditions. Concentrations of 1:500,000, 1:1,000,000, and 1:2,000,000 administered at weekly and biweekly intervals were tested. It was found that Lignasan administered at a concentration of 1:1,000,000 for a 1-hour exposure at biweekly intervals was particularly effective for the control of the unbranched filamentous green algae of the family Ulothrichaceae and adequate for the control of the branched filamentous green algae of the family Cladophoraceae. As the control of filamentous green algae is the principal problem in rearing ponds, Lignasan offers an excellent solution.

While tests of the algaecidal properties of Lignasan were being made, it was observed that chinook salmon (Oncorhynchus tshawytscha) were much more tolerant of the 1:1,000,000 concentration than of the 1:500,000 concentration. In further toxicity tests it was determined that chinook fingerlings would tolerate a 1:1,000,000 concentration for a 20-hour period with no mortality and no visual signs of distress. At a concentration of 1:500,000, distress was apparent after 1 hour and mortality developed after a 5-hour exposure. These toxicity tests were conducted in aquaria from which the fish were removed immediately at the conclusion of the test period. Such a procedure is not possible in pond treatment, although a drawdown of the water level of the pond prior to the conclusion of the treatment period and for 15 minutes after conclusion of the treatment does reduce the treatment concentration rapidly. Such a procedure is necessary if mortality from a 1-hour treatment with a 1:500,000 concentration of Lignasan in ponds is to be avoided.

The wide divergence in the toxicity of these two concentrations of Lignasan suggested that an alteration in the treatment method would increase the exposure period and possibly make the 1:1,000,000 concentration effective in the control of bacterial gill disease. Tests conducted

during 1957 indicated that a 1:1,000,000 concentration of Lignasan was effective both as a therapeutic and as a prophylactic for bacterial gill disease when altered treatment techniques were employed.

Three treatment methods are adapted to the use of Lignasan at a 1:1,000,000 concentration. In the first, the pond water is drawn down to the desired treatment depth and the required amount of Lignasan for a 1:1,000,000 concentration is introduced after the inflowing water has been shut off. The Lignasan may be introduced as the dry powder sprinkled over the surface of the water, or a dilution with water may be made and introduced by means of a sprinkling can. At the end of the 1-hour exposure period the normal inflow is resumed and the pond is allowed to fill to its normal operating level. No attempt to flush out the Lignasan is made either during or after treatment.

The second treatment method is applicable when the poundage of fish in the pond exceeds the capacity of the water volume, at the normal treatment depth, to support the fish for the full hour-long treatment period without danger of asphyxiation. In this method the pond is drawn down to the regular treatment depth, Lignasan sufficient for twice the volume is introduced at this water depth and, during the treatment period, the pond is allowed to fill at its normal inflow to twice its normal treatment capacity, after which the inflow is shut off until the 1-hour exposure period is completed. Again, the pond is allowed to fill to its normal operating depth without drainage after the inflow is turned on. As the fish will tolerate a 1:500,000 concentration of Lignasan for 1 hour and in this procedure they are subjected to a constantly diminishing concentration until the 1:1,000,000 depth is reached, no danger to the fish is involved. The carrying capacity at the increased treatment depth is, of course, doubled.

The third method is used principally in troughs and small tanks. In this procedure the fish are treated at or very close to the normal operating depth of the equipment. The inflowing water is shut off, Lignasan sufficient for a 1:1,000,000 concentration is introduced, and at the end of the 1-hour exposure the normal inflow is resumed. Here, again, no drainage takes place either during or after treatment.

All three treatment methods have been tested and found adequate for the control of bacterial gill disease. Prophylactics administered at biweekly intervals have controlled the disease in Foster-Lucas ponds. When the disease has been allowed to reach epidemic proportions, a single treatment has removed the bacteria. No unusual mortality has been encountered in any of the treatment methods when a 1:1,000,000 concentration of Lignasan has been used.

The substitution of a 1:1,000,000 concentration of Lignasan for the previously recommended 1:500,000 concentration and the alteration of the treatment technique whereby the exposure period is lengthened make Lignasan a safe, economical, and efficacious treatment for the control of bacterial gill disease. The bactericidal and algaecidal properties of Lignasan, in conjunction with its low initial cost, make it a most desirable addition to the methodology of salmon culture.

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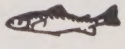

Filtering rates of the hard clam, Venus mercenaria L., have been determined by ascertaining the number of radioactive planktonic algae removed from water pumped through the filtering mechanism of the clam.

TO CONTRIBUTORS

Contributions for publication in THE PROGRESSIVE FISH-CULTURIST are always welcome. All contributions should be directed to: The Editor, THE PROGRESSIVE FISH-CULTURIST, Bureau of Sport Fisheries and Wildlife, Washington 25, D. C.

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